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The effects and fate of copper from pig slurry when applied to soil

by

J. Price

Thesis presented for the degree of
Doctor of Philosophy
in the Faculty of Science
of the University of Edinburgh

June 1979

DECLARATION

This thesis was composed by the author and the experimental sections, Chapters 2 to 6 inclusive are records of the work carried out by him on original lines of research.

The experimental work recorded in Chapter 7 was carried out in collaboration with Dr N. F. Suttle, Moredun Institute, Edinburgh. The author was responsible for suggesting this line of research initially and was involved on an equal basis with Dr Suttle in the planning and execution of the work.

All sources of information are shown in the text and all help given by other people is indicated in the acknowledgements.

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CONTENTS

ABSTRACT

Copper is widely used as a growth promoter at levels of up to 250 mg Cu/kg in rations for fattening pigs, resulting in the production of excreta containing high concentrations of this element. Since livestock excreta have traditionally been returned to the land as a fertiliser, concern has been expressed regarding the possible effects on the soil-plant-animal system of disposal of large amounts of copper-rich pig manures or slurries on agricultural land.

(a) The effect on copper content of herbage and accumulation of copper in soil following application of copper-rich pig slurry to pasture was investigated in field studies. Slurry produced by pigs receiving supplementary copper at a level of 200 mg Cu/kg D.M. in the ration contained up to 760 mg Cu/kg D.M. and was incorporated into soils prepared for seeding with ryegrass and white clover. The slurry copper, applied at a rate of 9 kg/ha increased the copper content of the mixed herbage by a maximum of 1.3 mg/kg D.M. on a light sandy soil; herbage Cu content was unchanged on an imperfectly drained clay-loam soil. Application of slurry copper at a similar rate to the established sward increased herbage copper contents by a maximum of 6.5 and 5.6 mg Cu/kg D.M. on the sandy and the clay-loam soils respectively. Copper in clover was increased to a greater concentration than that in ryegrass, the respective maximum concentrations in each species being 24.8 and 13.4 mg Cu/kg D.M.

Repeated applications of copper-rich slurry to the soil surface in an established ryegrass sward increased herbage copper content

from 4 to 16 and from 8 to 23 mg Cu/kg D.M. on the sandy and on the clay-loam soils respectively. Dilution of the slurry with water resulted in greater increases in herbage copper than did undiluted slurry.

The addition of up to 30 kg slurry Cu/ha over a 2 year period markedly increased the level of EDTA-extractable copper in the soils; it was estimated that 89 to 90% of slurry copper applied was extractable from the topsoil using this reagent.

(b) Less than 5 mg Cu/l were found in the aqueous phase from slurry, while a fine particulate plus colloidal fraction in the solids contained in excess of 2000 mg Cu/kg D.M. Evidence was obtained suggesting that copper in the dried slurry solids was associated with the organic matter and was not present as the insoluble sulphide. Copper in the aqueous phase from slurry was found to increase the concentration of copper in ryegrass.

(c) Carbon mineralisation in soil was unaffected by addition to the soil of a fraction of slurry solids containing 2278 mg Cu/kg D.M. which increased the total copper content of the soil by 108 mg/kg.

(d) The true availability (4.3%) to sheep of copper in dried slurry solids was found to be similar to that of copper fed as $CuSO_4$. The availability (5.8%) to sheep of copper in herbage grown on slurry treated land was significantly greater than that (4.8%) in herbage from untreated land.

(e) The potential hazards to crops and livestock of disposal of high copper pig slurries on agricultural land are discussed.

INTRODUCTION

The inclusion of high concentrations of copper in the rations for fattening pigs has become common practice in the United Kingdom and the effectiveness of this element as a growth promoter in pigs maintained under normal farm conditions has been reviewed by Braude (1965, 1975). One of the main advantages of copper as a growth promoter is that of low cost combined with proven effectiveness. However, very little of the ingested copper is absorbed by the pig and as a result the faeces contain high concentrations of this element which, unlike the organic matter, cannot be degraded.

Traditionally, the fertility of land has depended to a large extent on the addition of animal wastes to the soil. Although inorganic fertilisers have made it possible to grow forage and arable crops without the need for organic manures, virtually all excreta produced by livestock in the United Kingdom continue to be returned to the land either as fertiliser or simply as a means of disposal. It is hardly surprising therefore that in view of the high concentrations of copper in piggery waste as at present constituted, concern has been expressed over the possibility of adverse ecological consequences arising from continued application of this material to agricultural land.

The disposal of sewage sludge on agricultural land has been shown to result in enhanced copper levels in crops (Lagerwerff, 1967, Le Riche, 1968) and it has become evident that contamination of soil with copper and indeed other metals, arising from continued heavy applications of this material, may be permanent (Purves, 1972). Copper-rich pig slurry has been shown in short-term trials to increase the concentration of copper in herbage and the level of extractable copper in soils (Batey, Berryman and Line, 1972). There is, however, little information available at present regarding either the extent to which slurry copper will accumulate in soils or the risk of crop production being adversely affected by enhanced levels of plant-available copper in soils as a consequence of repeated applications of pig slurry over a period of years.

Accumulation of heavy metals in soils, in addition to constituting a potential toxicity hazard to plants, may affect soil microbial activity. Tyler (1975) has reported that nitrogen mineralisation may be adversely affected by copper concentrations greater than 50 mg/kg air dry soil; no information on the effect of slurry copper on microbial activity in soils has as yet been reported.

Since sheep are particularly susceptible to high intakes of copper, any factor resulting in large increases in the copper content of feedstuffs must be regarded as a potential toxicity hazard to this species. Feenstra and Van Ulsen (1973) have reported that hay which had been accidentally surface-contaminated with copper-rich pig slurry was the cause of a number of deaths in sheep fed this material. Information regarding availability to livestock of copper in slurry and crops grown on slurry treated land is therefore required in order to assess the toxicity hazard from copper in these sources.

This thesis contains a report of investigations carried out to assess the hazards outlined above. In field experiments, the long term effects of disposal of copper-rich slurry on accumulation of the metal in soil and the resultant effects on herbage copper concentrations have been studied. The availability of added slurry copper in soil has been estimated empirically by chemical extraction and the effect of slurry copper on soil microbial activity has been assessed in the laboratory. The true availability of copper in slurry and in herbage from slurry treated land has been estimated for sheep.

The relevant literature has been reviewed and the results of experimental work discussed in relation to current agricultural practice.

CHAPTER 1.

REVIEW OF LITERATURE

1.01. The essentiality and toxicity of trace elements in living organisms.

Many mineral elements occur in tissues of living organisms in such small amounts that the early workers were unable to measure their precise concentration with the analytical methods then available. These elements, reported as occurring in traces, became known as trace elements and were regarded as a form of contamination since no known function could be assigned to them.

As methods of analysis and experimental techniques improved, increasing numbers of these elements have been shown to be essential in minute quantities to living organisms. According to Cotzias (1967), a trace element may be designated as "essential" if it meets the following criteria:-

(i) it is present in all healthy tissues of all living things,

(ii) its concentration from one organism to the next, within a species, is fairly constant,

(iii) its withdrawal from the environment of the organism induce reproducibly, the same structural and physiological abnormalities,

(iv) its addition either prevents or reverses these abnormalities,

(v) the abnormalities induced by deficiency are always accompanied by specific biochemical changes,

(vi) these biochemical changes can be prevented or cured when the deficiency is prevented or cured.

The trace elements have thus been classified as essential or non-essential, any further classification being difficult since the only characteristic that is common within either group is their occurrence in the tissues of microorganisms, plants and animals.

The elements boron, chlorine, cobalt, copper, chromium, iron, fluorine, iodine, manganese, molybdenum, nickel, selenium, silicon, tin, vanadium and zinc are now recognised as essential for at least one of the above life-forms (Schwarz, 1977). Each element, however, may not be necessary for all life-forms. Higher plants, for instance, have not as yet been shown to have a requirement for cobalt while boron does not appear to be essential to animals (Sauchelli, 1969). As research on trace elements continues, it seems likely that the list of those known to be essential will be extended. It would now appear from recent studies that arsenic may well be essential for mammals (Anke, Grün, Partschefeld, Groppel and Hennig, 1978).

The requirement of plants for copper did not emerge as an isolated, clear-cut discovery. Grossenbacker (1916) and Floyd (1917) described the use of Bordeaux fungicide, a copper sulphate-lime preparation, to cure diseases of fruit trees. The effect of the copper was at that time attributed to protection of the plant against microorganisms. Allison, Bryan and Hunter (1927) and Felix (1927) obtained increases in crop yield on soils with a high organic matter content by using heavy applications of copper sulphate. At first all increases in yield with copper were ascribed to chemical stimulation (Kanda, 1904) and it was not until the appearance of the publications of Sommer (1931) and Lipman and MacKinney (1931) that a nutritional interpretation was applied. Sommer reported decreases in yield of up to 90 per cent as a result of omitting copper from the nutrient solution for three species of plant grown by water culture while the latter workers found that barley would not produce seed when grown in nutrient solution containing less than 0.06 mg Cu/l. Thus the first suggestions were made that higher plants require copper for normal growth, development and reproduction. The essentiality of copper for plants was soon verified by a number of other workers including Arnon & Stout (1939), Hoagland (1940) and Piper (1942) using various plant species including grasses.

Conclusive evidence for the essentiality of copper for animals emerged from studies on the regeneration of haemoglobin in the rat (Hart, Steenbock, Waddell and Elvehjem, 1928). Haematopoesis was considered to be the major physiological activity in which copper was involved until field studies revealed other functions for this element. A number of disorders of grazing sheep and cattle (reviewed, Underwood, 1971) were found to be due to a dietary deficiency of copper or to respond to copper therapy. Defects in the processes of pigmentation, keratinisation of wool and hair, bone formation, reproduction, myelination of the spinal cord, cardiac function and connective tissue formation in addition to those of growth and haematopoesis were found to be manifestations of copper deficiency. From the results of feeding trials, the Agricultural

Research Council, London (A.R.C.) have suggested that the minimum requirements of sheep and cattle for copper (A.R.C., 1965) are 5 and 10 mg Cu/kg dietary dry matter (D.M.) respectively and that rations containing 50 mg Cu/kg D.M. are likely to meet the copper requirements of all classes of swine (A.R.C., 1967).

While a supply of the essential trace elements may be required by living organisms, many, if not all of these elements are potentially toxic. Acute oral toxicity tests using the laboratory rat and summarised by Bowen (1966), have shown that copper is as toxic as cadmium; that iron and molybdenum are more toxic than lead and that the toxicity of mercury is surpassed by that of vanadium and selenium, both of which are proven to be essential.

Reviews by Beran (1965) and Coombs (1965) have dealt with the importance in agriculture of the bacteriocidal, fungicidal and molluscicidal properties of copper salts. Although any of the heavy metals may be toxic to plants at some level of solubility, only a few have been widely observed to cause phytotoxicity when in soils. Because of the precipitation and sorption of most metals by soils (Hodgson, 1963; Allaway, 1968; Jenne, 1968 and Lindsay, 1974) mainly only copper, nickel and zinc toxicities have occurred frequently, other elements such as chromium and tin being toxic in solution culture but not phytotoxic in soils even at very high levels (Foy, Chaney and White, 1978). Lead and cadmium, although non-essential trace elements, have received considerable attention, not because of their phytotoxicity but because plant uptake is an important point of entry into the food chain (Mahaffey, Corneliussen, Jelinek and Fiorino, 1975).

The most general symptoms of phytotoxicity due to excesses of the heavy metals Cu, Ni and Zn are stunting of growth and chlorosis similar to that induced by iron deficiency. Although chlorosis and the interactions of toxic metals with iron have been repeatedly demonstrated (Hewitt, 1952; Reuther and Smith, 1954; Chapman, Bradford and Rayner, 1969; and Struckmeyer, Peterson and Hsi Mei Tai, 1969) the physiology and biochemistry of Cu, Ni and Zn toxicity have received little study beyond the responses of intact plants (Hewitt, 1948; Smith and Specht, 1953; De Kock, 1956, and Spencer, 1966).

The subject of trace element toxicity in relation to plant physiology has been included in reviews by Bollard and Butler (1966), Brown and Jones (1975); Chaney and Giordano (1977), and Foy, Chaney and White (1978). The tolerance of plant species of importance in agriculture to high levels of copper and zinc in soils and factors affecting uptake of these elements by crops is discussed in greater detail in a later section of this chapter.

From the comprehensive review by Underwood (1971) of trace elements in human and animal nutrition it is apparent that these elements, essential and non-essential, are potentially toxic if dietary intakes are excessively high. It is also apparent that there are important species differences in tolerance to ingestion of excessive amounts of a particular element. Thus, in reviewing heavy metal toxicities, Bremner (1974) has stated that chronic copper poisoning does not represent a major toxicological problem in human medicine whereas Todd (1969) has reported that chronic copper poisoning is relatively common in ruminant livestock and of appreciable economic importance. Monogastric animals appear to be very much more tolerant of high copper intakes than ruminants. The rat, for example, has been shown to grow normally and remain healthy when fed diets containing 500 mg Cu/kg D.M., about 100 times normal (Boyden, Potter and Elvehjem, 1938), whereas Bracewell (1958) has reported that rations containing less than 50 mg Cu/kg D.M. may result in copper poisoning of housed sheep.

Davies (1974) has stated that "some of the toxic effects resulting from the ingestion of excessive amounts of both essential and non-essential elements may be ascribed to interactions with biological processes directly or indirectly involving other trace elements". Thus, the symptoms of chronic molybdenum toxicity in the ruminant animal are manifestly those of copper deficiency, sulphur playing an important role in this antagonistic interaction (Dick, 1954; Suttle, 1974; Suttle and MacLauchlan, 1976; Ward, 1978). It should be noted, however, that the symptoms of acute molybdenum toxicity (molybdenosis) arising from massive intakes of this element are quite different to those of the chronic disorder and of copper deficiency (Ferguson, Lewis and Watson, 1938; Ward, 1978).

The potential hazard to swine, cattle and sheep of excessive dietary intakes of copper and zinc specifically will be discussed in greater detail in later sections of this Chapter with regard to tolerance of each species to these elements and to their interactions with other trace elements in the diet.

1.02. The biochemical basis of action of trace elements in living organisms.

Almost forty years ago, Green (1941) stated "that enzymic catalysis is the only rational explanation of how a trace of some substance can produce profound biological effects". The truth of this statement has been abundantly demonstrated with respect to the essential trace elements. The involvement of these elements in enzymic reactions ranges from weak, ionic strength effects to highly specific metal-protein associations, the metalloenzymes (Schutte, 1964).

In the case of the metalloenzymes, the metal is firmly associated with the protein and cannot be removed from this association by dialysis. Removal of the metal by more drastic means results in loss of enzymic activity which cannot readily be restored either by readdition of the metal or by any other metal (Vallee and Wacker, 1970). The molecular mechanisms involving the metalloenzymes and the nature of the metal ion specificity have been illuminated by sophisticated studies involving amino acid sequence determinations, chemical modification and X-ray crystallography. Thus Vallee, Riordan and Coleman (1963) in a study of carboxypeptidase A, a zinc dependant enzyme active in peptide bond hydrolysis in animals, proposed a transition state structure for the enzyme-substrate complex involving the Zn^{2+} ion bound in part to the enzyme by a specific sulphydryl group.

The metal-ion-activated enzymes show a lower specificity for metal ions, these ions being less tightly bound to the enzymes and Malmstrom and Rosenberg (1959) have suggested that these ions may function by (1) inducing or maintaining the active confrontation state of the enzyme protein molecule (2) acting as a bridge in the formation of a ternary complex, a purely co-ordinate role (3) changing the electronic structure of the substrate molecule.

In recent years, increasing numbers of trace element-enzyme associations have been identified and related to the manifestations of deficiency or toxicity states in plants and animals. The essentiality of copper for the mammal was first recognised in 1928 by Hart et al who reported that the copper deficient animal became seriously anaemic. This anaemia has now been related to a low blood serum level of the copper-containing enzyme caeruloplasmin, which appears to control iron transport, necessary for haemoglobin synthesis, by virtue of its ferroxidase activity thus providing a link between copper and iron metabolism (Frieden, 1971; Evans and Abraham, 1973).

The trace metal ions may form either ionic or covalent complexes, though with different affinities, with a multiplicity of electron donor atoms that are present, mainly as derivatives of nitrogen, sulphur and oxygen, in cellular components. Such interactions of toxic metallic ions (Webb, 1977), although unlikely to be specific for either cation or ligand are strong and may alter molecular conformation, break hydrogen bonds, displace functional cations from their action sites and inhibit catalytic activity.

Metabolic targets of toxic metals therefore abound in living tissues and it is not surprising that there are many disorders of plants and animals arising from trace element deficiencies and excesses, for which there are as yet no complete explanation in biochemical or enzymic terms. For example, the chlorosis in many plants caused by excess copper (Chapman, Liebig and Vanselow, 1939; Agarwala, Bisht and Sharma, 1977) may be partly explained by iron deficiency as a result of copper inhibiting translocation from roots to leaves (Lingle, Tiffin and Brown, 1963; Wallace and de Kock, 1966). The details of this copper-iron interaction in the root are not yet known.

1.03. The copper requirement of pigs.

The minimum copper requirements of pigs for growth or reproduction and lactation cannot be stated with any precision. In a comparison of dietary copper levels of 6, 16 and 106 mg/kg D.M. Ullrey, Miller, Thompson, Zutaut, Schmidt, Ritchie, Hoefer and Leucke (1960) obtained no significant differences in growth rate or in the feed:gain efficiency ratio (F.G.E.) of young pigs.

These dietary copper concentrations were found to maintain blood haemoglobin levels and plasma copper concentrations within the normal range.

The Agricultural Research Council of Great Britain (A.R.C., 1967) in reviewing studies involving various dietary copper concentrations for pigs concluded that, while 4 or 6 mg Cu/kg D.M. may be adequate for growing pigs, dietary copper concentrations around 50 mg/kg D.M. should be adequate for growth, reproduction and lactation. In support of these A.R.C. conclusions a recent investigation of copper and iron supplements on the performance and haematology of sows and their progeny through four reproductive cycles may be cited. Lillie and Frobish (1978) reported that a dietary copper concentration of 19.6 mg/kg D.M. may be adequate for growing and breeding pigs in terms of haematology, while a slight improvement in growth rates was evident when dietary copper was increased to 64.6 mg/kg D.M.

1.04. Copper as a growth stimulant in pigs.

As long ago as 1928, Evvard, Nelson and Sewell demonstrated an improvement in the growth of pigs when copper sulphate was added to their normal rations. Later, Carpenter (1946) reported growth improvements following addition of 112 mg Cu/kg D.M. to the rations of pigs as a treatment for digestive upsets.

Braude, in 1945, observed pigs licking copper fitments which were often in contact with urine on the floor of the pig pens. Such was the craving of the pigs for copper that none of these fitments were left after twelve months. Subsequently, tests with a number of licks containing various metals demonstrated that copper was the only metal in which the pigs were interested (Braude, 1948). The question then arose as to the nutritional significance of these observations. Further research on the effects of high copper intakes in relation to the growth performance of pigs was, however, delayed for a number of years in view of the claim in most text books at that time that dietary copper concentrations greater than 30 to 50 mg/kg D.M. were toxic (Braude, 1965).

In the years 1955 to 1957 a number of reports were produced by workers at the National Institute for Research in Dairying, Shinfield, England, demonstrating that significant increases in growth rate could be obtained by addition of 250 mg Cu/kg D.M. to the rations for growing pigs (Barber, Braude and Mitchell, 1955a, 1955b; Bowler, Braude, Campbell, Craddock-Turnbull, Fieldsend, Griffith, Lucas, Mitchell, Nickalls and Taylor, 1955; Barber, Braude, Mitchell, Rook and Rowell, 1957). These reports have since been followed by numerous others from various parts of the world, verifying the growth stimulatory effect of copper supplementation of diets for fattening pigs (Bellis, 1961, Bunch, Speer, Hays, Hawbaker and Catron, 1961, 1963; Castell and Bowland, 1968; Kline, Hays and Cromwell, 1971).

A summary of the results of all the trials reported in the literature up to mid-1965 in which the performance of growing pigs receiving a copper sulphate supplement in the ration supplying 250 mg Cu/kg D.M., was directly compared with that of similar control animals receiving no copper supplements has been reported in a review by Braude (1965). The average improvement associated with a supplementation level of 250 mg/kg D.M. was reported to be +8.1% (from -12% to +25%) in daily live weight gain and +5.4% (from -5.2 to +12.6%) in efficiency of feed utilisation. In a later review Braude (1975) has summarised the results of a further 119 trials carried out between 1965 and 1975 with 250 mg/kg supplementary copper, the increases in liveweight gain and feed:gain ratio in these trials being 9.1% and 7.4% respectively. There would therefore appear to be little doubt that copper supplementation of pig fattening rations increases the efficiency with which the pig utilises its feed and that this is reflected in increased rates of live weight gain.

In addition to the use of copper sulphate in pig rations at a rate of 250 mg Cu/kg D.M., higher and lower rates of addition have been examined in relation to growth rate and accumulation of copper in the liver of the pig. From the results of Bunch et al 1961 and Hawbaker, Speer, Hays and Catron (1961) it would appear that the response in growth rate to copper added to the ration at a rate of 375 mg/kg D.M. is slightly lower than that obtained at 250 mg/kg D.M. At a level of addition of 500 mg Cu/kg D.M. there is a danger of excessive copper accumulation in the liver of the pig (Lucas and Calder, 1957; Bunch, McCall, Speer and Hays, 1962). The results of trials carried out using supplementary copper in the rations at 125 mg/kg D.M. have been summarised by Braude

(1965); the response in liveweight gain at this level of supplementation was approximately half that obtained at 250 mg Cu/kgDD.M. It would therefore appear that the level of supplementary copper for the optimum growth promotion effect in fattening pigs is around 250 mg/kg in the ration.

The maximum allowable concentration of copper in animal feedstuffs was the subject of a Directive in European Economic Community Secondary Legislation produced in 1972 (E.E.C. Directive 1972). Application of this Directive, which stipulates that the maximum allowable copper concentration in animal feedstuffs should not exceed 125 mg/kg D.M. would, on the basis of the experimental work reviewed above, seriously reduce the potential beneficial effect of high level copper supplementation of rations for growing pigs. This Directive has not as yet been enforced.

Several investigations have been carried out to ascertain whether it is the copper ion or the sulphate radical in copper sulphate which is responsible for the growth promotion effects. Sodium sulphate has been shown to be ineffective (Hawbaker, Speer, Jones, Hays and Catron, 1959) but soluble copper compounds such as the carbonate (Allen, Barber, Braude and Mitchell, 1961; Bunch et al, 1962) and chloride (Hawbaker et al, 1959) were as effective as the sulphate. Copper II oxide, which is insoluble in water, has been shown to be less effective as a growth promoter than water-soluble forms of copper (Bunch, Speer and Hays, 1960; Bunch et al, 1961) while copper II sulphide, also water-insoluble, apparently has no growth promoting effect (Cromwell, Hays and Clark, 1978). Since copper sulphide would be insoluble and the oxide slowly solubilised in the acid medium of the pigs stomach, it would appear that copper ions are required at some point in the pigs digestive system for the growth promoting effect.

There is as yet little indication in the literature as to the mode of action of copper in promoting growth in the pig when added to the rations at levels many times greater than the recognised dietary requirement of the animal for maintaining normal body functions. Additions of cobalt, iron, manganese and nickel at similar levels have been found to be without effect (Evvard et al, 1928; Giessler and Kirchgessner, 1959; Hoefer, Miller, Ullrey, Ritchie and Leucke, 1960; Wallace, McCall, Bass and Coombs, 1960). Furthermore, the reports regarding the poor or negligible responses obtained with copper oxide or sulphide would suggest that a soluble copper salt or one which would be at least partly solubilised by the digestive juices, is necessary for growth promotion.

Although there has been speculation that copper may be acting as an antibiotic on bacteria in the gut, Williams-Smith and Jones (1963) have reported that high-copper pig rations neither suppress nor increase bacterial multiplication in any section of the alimentary tract. These workers have pointed out however, that the effect might possibly be on bacteria which they have been unable to culture or differentiate from other types present.

The work of Andrla (1964) on hydrogen sulphide-producing bacteria in pig faeces has been taken (Braude, 1965) to provide some support for the suggestion that copper may act in removal of the poisonous hydrogen sulphide produced in the gut through precipitation of cupric sulphide. This hypothesis would however appear to be untenable in view of the finding (Cromwell, Hays and Clark, 1978) that sodium sulphide, which would produce free sulphide ions or hydrogen sulphide in the gut, does not in any way reduce the growth response to copper.

Whatever its mode of action, copper is now a proven growth promoter for the pig and Braude (1965, 1975) has summarised the responses in relation to economic benefit to the pig industry in the United Kingdom.

1.05. Absorption, tissue distribution and excretion of copper in the pig.

Absorption and excretion of copper in the pig have been little studied despite the widespread use of this element at high concentrations in fattening rations. Bowland, Braude, Chamberlain, Glascock and Mitchell (1961) have used ^{64}Cu labelled copper sulphate and sulphide to estimate the amounts of copper in these forms, which is absorbed from rations containing either 40 or 250 mg Cu/kg D.M. When added to the diet as the sulphate, approximately 9% of the ingested ^{64}Cu was absorbed from the diet containing 40 mg Cu/kg, while only 3.8% was absorbed from the high-copper diet. Approximately 2% of ^{64}Cu added to the diets as sulphide was absorbed at 40 and 250 mg total copper/kg D.M. in the diets. Kirchgessner, Weser and Friesecke (1963) later reported that the retention of copper by pigs fed diets containing 250 mg Cu/kg D.M. was extremely low.

The addition of up to 150 mg Cu/kg D.M. to pig rations has been shown to result in small changes in the copper concentration in the liver of pigs. Thus, Kline, Hays and Cromwell (1971) reported liver copper concentrations of 16 and 19 mg/kg D.M. for pigs fed rations containing 0 and 150 mg Cu/kg respectively. When the level of supplementation is increased to 500 mg/kg D.M. liver copper concentrations may exceed 4000 mg/kg D.M. (Cassidy and Eva, 1958; Allcroft, Burns and Lewis, 1961). It would appear from the work of Allen and Harding (1962) that clinical symptoms of copper toxicity may develop when liver copper reaches this high concentration. Although copper poisoning of pigs has been reputed to have occurred in pigs fed diets containing up to 250 mg Cu/kg (Gordon and Luke, 1957), the basis for diagnosing copper poisoning was rather dubious (Braude, 1965). Copper accumulation in tissues of the pig, other than the liver and to a certain extent, the kidney, does not occur (Kline et al, 1971, Cromwell et al, 1978).

It is interesting to note that while dietary molybdenum and sulphate have been shown to reduce copper storage in the liver of ruminants (Dick, 1954), no such effect has been found in the pig (Kline, Corzo, Hays and Cromwell, 1973).

Of the dietary copper absorbed by the pig, Bowland et al (1961) found that less than 10% was excreted in the urine.

Since less than 10% of the ingested copper is actually absorbed, whether from a copper supplemented or unsupplemented ration, approximately 90% of the dietary copper will be excreted in the faeces.

Thus the feeding of high-copper pig rations will result in the production of manures or slurries containing high concentrations of this element. (Slurry is a mixture of all the urine and faeces together with varying quantities of water, and without the addition of bedding materials).

The addition of copper to pig rations has in some cases been held responsible for outbreaks of parakeratosis in pigs (O'Hara, Newman and Jackson, 1960). This skin disorder had earlier been found to respond to treatment with zinc either applied externally or included in the rations (Tucker and Salmon, 1955). The inclusion of 150 mg Zn/kg in pig rations has been shown to prevent the occurrence of parakeratosis and to reduce copper storage in the liver when rations contained up to 450 mg Cu/kg (Suttle and Mills, 1966). As a result, zinc is now routinely included in high-copper pig rations at a level of 100 to 150 mg/kg D.M. and zinc concentrations of 700 to 1000 mg Zn/kg D.M. have been reported (A.R.C., 1976a). in slurries produced by fattening pigs.

Data from a number of studies on pig slurry in Great Britain have been examined and the reported copper concentrations, expressed on a dry matter basis for uniformity, are shown in relation to the level of supplementary copper in the pig rations in Table 1.1. The ratio of copper in slurry to dietary

TABLE 1.1.

Summary of the copper concentrations reported in the literature for pig slurry in relation to copper content of the pig rations fed.

Concentration of Copper (mg/kg D.M.) (A) Ration	(B) Slurry	B/A	Reference	
200	856	4.28	Batey et al	(1972)
*	643-1575	-	Batey et al	(1972)
250	675	2.70	Berryman	(1970)
250	737	2.95	Dalgarno & Mills	(1975)
150	613	4.09	Hendrosoekarjo & Pearce	(1978)
180	325	1.81	Kneale & Smith	(1977)
250	1291	5.16	Lawrence & Gibbs	(1973)
60	153	2.55	McGill et al	(1975)
328	1422	4.34	Robinson et al	(1971)
*	160	-	Woodside	(1973)

*, dietary copper level not reported.

copper appears to vary considerably from one slurry source to another. Since pig fattening rations in Great Britain are fairly uniform being largely based on barley grain with fish or soya bean meal as a source of additional protein (Hobson and Robertson, 1977), these variations might be partly explained by differing losses of organic matter from the slurry due to decomposition of organic matter during storage, the conditions of which were not reported.

A search of the literature has revealed only two references regarding the form in which copper is present in slurry from pigs fed copper supplemented rations. Andrla (1964) has suggested that reduction of copper to the insoluble sulphide by hydrogen sulphide of bacterial origin may occur in the digestive tract. The observation that the faeces of copper supplemented pigs were very dark, almost black, in colour was taken to support the copper sulphide hypothesis. Robel and Ross (1975) have presented further evidence interpreted as supporting the sulphide hypothesis. A soluble copper fraction was extracted from slurry produced by pigs fed rations containing 500 mg Cu/kg D.M. using 0.3 N hydrochloric acid in which copper II sulphide was shown to be insoluble. The insoluble slurry residue was then digested in an oxidising mixture containing perchloric acid which had been shown to completely dissolve copper II sulphide. Approximately 36% of the copper in slurry was found to be HCl-soluble and therefore not present as the sulphide. The remaining 64% which was solubilised after oxidation and treatment with 0.3 N HCl was concluded to have been present mainly as the sulphide with the possibility that a small proportion could have been in the form of the water-insoluble carbonate or phosphate. These workers also stated that copper present in slurry as the sulphide would "lessen the contamination problem".

1.06. Utilisation and disposal of pig slurry.

Prior to commercial manufacture of synthetic fertilisers, animal waste products, especially animal faeces, were highly valued for use as fertiliser materials. Animal and plant residues were returned to the soil to enhance production of another crop, which in turn could be returned to the food chain. Pig slurry in common with excreta from other farm livestock, contains appreciable quantities of the major plant nutrients nitrogen, phosphorus and potassium (McAllister, 1963; Berryman, 1970). The importance of the proper conservation and use of animal wastes has been emphasised in reports by the Department of the Environment (1974) and the Joint Consultative Organisation for Research and Development in Agriculture and Food (1974) in Great Britain.

Although there has been no national farm survey of methods of disposal of pig slurry or other livestock excreta in Great Britain, a recent report regarding studies on livestock wastes (A.R.C., 1976a) has indicated that, on most farms animal waste is recycled as a fertiliser for grassland and some arable crops. Estimates of the fertiliser value of pig slurries and recommendations as to suitable rates of application for various crops have been given in advisory leaflets produced by the Ministry of Agriculture, Fisheries and Food for England and Wales (MAFF, 1969, 1976). It has thus been suggested (MAFF, 1976) that for silage, grazing and cereal production the number of pigs, the manure from which would supply the approximate maximum nutrient requirement of 1 hectare of land for 1 year, would be

80, 35 and 17 respectively.

Jelenek (1977) has quoted average values for the total solids produced in urine and faeces by pigs of various live weights up to 100 kg. Since fattening pigs in Britain are normally slaughtered at 90 to 100 kg live weight, it is assumed that the average live weight of these pigs nationwide is a maximum of 50 kg, producing a quoted 6.3 kg of excreta daily with a 12% D.M. content. Thus 80, 35 and 17 pigs would produce approximately 11, 5 and 2.5 tonnes of solids per year.

Increasing demands for animal products for human food (Barreveld, 1977) have led to intensification of animal production with production units tending to specialise in one type of animal. In simple terms, this has meant greater numbers of livestock being carried on the same or less land in comparison to traditional farming practice. From 1960 to 1974 the total number of pigs in Great Britain increased from 2.5 to 7.7 million while the number of herds fell from 110 to 39 thousand (A.R.C., 1976b).

With this increase in intensification came problems in disposing of the excreta produced. An examination of the information in Agricultural Statistics for England and Wales (MAFF, 1977) has shown that in 1975, 1.9% of the pig herds were maintained on holdings with no land at all for disposal of the excreta. A further 8.1% of the herds with an average of 326 pigs per head were on holdings less than 2 hectares (ha) in size, a situation where the excreta produced would have a fertiliser value approximately four times greater than the maximum major nutrient requirement of the land available if completely under grass.

The collection and storage of pig excreta as a slurry in modern systems of housing greatly reduces labour costs and ensures practically complete recovery of faeces and urine thereby reducing the risk of polluting water courses caused by drainage direct from the farm yard (McAllister, 1972). Pollution of water courses resulting from direct run-off from the soil surface or percolation of nutrients through the soil following heavy applications of slurry may also be minimised from a knowledge of the hydraulic loading capacity of the soil (O'Callaghan, Pollock and Dodd, 1973). However, in the case of cattle, pig and poultry production units with little or no land for use of the material as a fertiliser, methods other than the direct application of the high water content slurries to soil had to be sought. The literature regarding physical and biological treatment of these wastes is extremely extensive and will not be reviewed here since the methods have been summarised recently by Hobson and Robertson (1977). The main objectives of the various treatment systems are a reduction in the water and organic matter content of the slurries in order to produce a material which may be used as a fertiliser in as non-polluting a form as possible. A reduction in water content of the slurries may facilitate transport of the material for utilisation on other farms. Adverse effects on crop yield arising from anaerobic conditions in soils following heavy applications of untreated slurries (Burford, 1976) may be avoided by reducing the organic matter content and hence the Biological Oxidation Demand (B.O.D.) of the material before it is added to the soil.

The technical, economic and environmental aspects of the various methods of handling, disposal and utilisation for nutrient or energy recovery have been delineated and evaluated in a collection of papers published under the title "Animal Wastes" (1977).

In addition to the risk of polluting soils and water courses through the application of excessive amounts of N, P, K and readily degradable organic matter in pig slurries, copper and zinc present in this material may possibly have important environmental consequences. Although no figures are available regarding the total usage of copper as a growth promoter for pigs in Great Britain, the amount of copper added to soils yearly in slurry may be estimated.

Earlier in this section it was estimated that fattening pigs would on average produce excreta containing 0.138 tonnes D.M./head/year. Since the total fattening pig population in Great Britain was reported by the Central Statistical Office (C.S.O., 1979) to be 6.9 million in 1977, the total production of excreta for that year would be 0.95 million tonnes D.M. From a copper concentration of 675 mg/kg D.M. reported by Berryman (1970) for slurries produced by pigs receiving 250 mg Cu/kg in the ration, a total of 640 tonnes of copper would be added yearly to soils. If this amount of copper were to be distributed evenly over the 12 million hectares under grass or crop in Great Britain, the yearly addition of copper per hectare would be negligible. The question must, however, be asked as to how much slurry copper is added yearly when the limited area of land available to some intensive pig units is used simply as a disposal area with no regard to the fertiliser value of the waste. Physical and biological waste treatments, while useful in reducing the water and organic matter content of slurry, would have a concentrating effect as far as the copper is concerned. Waste treatments cannot alter the total output of copper in the waste and there is the possibility that these treatments may allow greater amounts of copper to be added to the land than would have been possible with untreated slurry where volume may have been a limiting factor.

At present there are no published statistics regarding the amounts of slurry copper added to soil on pig farms with limited areas of land for waste disposal. Earlier in this section it was pointed out that the average number of pigs on holdings with less than 2 hectares of land for waste disposal was 326 in 1975. Using the same data as for calculation of total slurry copper output per annum in Great Britain, the excreta of 326 pigs fed rations containing 250 mg Cu/kg, would result in the yearly addition of 30 kg copper to the 2 hectares of land. Batey, Berryman and Line (1972) have reported that 12 kg Cu/ha have been added to the soil yearly for three consecutive years on a pig farm in England. Such a rate of addition would enhance the total copper content of the topsoil by approximately 12 mg Cu/kg, which is just over half the total copper content, 20 mg/kg, reported for uncontaminated soils under cultivation (Vinogradov, 1959).

It is interesting to note that the pressure to find new methods for disposal of animal excreta has encouraged research into the recycling of such materials in rations for farm livestock. The nutritional value of dried animal wastes has been investigated in a number of studies where excreta, including pig faeces, were added to rations fed to ruminants (Bucholz, Henderson, Thomas and Zindel, 1971; Tinnimit, Yu, McGuffy and Thomas, 1972), pigs (Diggs, Baker and James, 1965) and poultry (Hodgetts, 1971, Nesheim, 1972). From a review of studies on recycling animal wastes in feedstuffs (Bhattacharya and Taylor, 1975) it would appear that rations containing 20 to 30% dried pig or poultry excreta may give satisfactory growth performance in cattle and sheep, ruminants being more effective utilisers of animal wastes than monogastric animals. If pig excreta containing 675 mg Cu/kg D.M. were included in rations at this level, the concentration of copper in the final ration would be increased by 135 to 202 mg/kg.

It is therefore not surprising that concern has been expressed as to the consequences of recycling copper-rich pig slurries on the soil-plant-animal system. The risk of slurry copper adversely affecting soil microorganisms, plants and animals will, however, depend on a number of factors such as the form in which copper is present in treated and untreated slurry, soil conditions and the extent to which this element gets into the food chain through uptake by plants or deliberate addition to ruminant diets by man.

1.07. Contamination of soils with copper and zinc.

The sources of trace element contamination of soils are numerous. Purves (1977) has outlined a number of sources giving rise to enhanced concentrations in the soils of urban and industrialised areas. These include fallout from atmospheric pollution, wind-blown dusts, corrosion of discarded metal objects and the deliberate addition to soil of waste materials such as soot, fuel ash, sewage sludge and household refuse. In comparing urban with uncontaminated rural soils, Purves (1970) found that mean levels of Ethylenediamine-tetra acetic acid (EDTA) extractable copper and acetic acid-extractable zinc in the former soils were 33 mg Cu/kg and 64.5 mg Zn/kg while the uncontaminated rural soils had mean levels of 11.5 mg Cu/kg and 11.3 mg Zn/kg.

Contamination of soils with copper and zinc in agricultural areas has occurred with the use of compounds of these elements as fungicides and to correct deficiencies in the soils. Reuther and Smith (1954) have reported that accumulation of copper occurred in soils in a number of orchards and vineyards in Florida as a result of the use of Bordeaux mixture and additions of copper directly to the soil. In this case excessive amounts of copper had been applied over a number of years and eventually phytotoxicity occurred. Similarly, zinc oxysulphate used in peach orchards adversely affected the growth of soya bean and cotton on these soils when the peach trees were removed (Lee and Page, 1967).

At present there is little information available regarding the accumulation of copper or zinc in soils as a result of pig slurry disposal. Batey et al (1972) have found that the application of 55,000 litres/ha. of pig slurry containing 12 kg of copper increased the EDTA-extractable copper content of the topsoil from 2.1 to 7.3 mg/kg, approximately half of the added copper being in an extractable form. This addition of slurry copper increased the copper content of the ryegrass sward from 10.7 to 17.7 mg Cu/kg D.M. These authors have also reported that in 3 years a total of 36 kg Cu/ ha were added to the soil in pig slurry on a farm in England, increasing the level of extractable copper in the soil by 50%. It would therefore appear that the effect of slurry copper on soil EDTA-extractable copper may vary depending on the soil or the slurry applied. However, it is worth noting that the growth of clover may be adversely affected when soil EDTA-extractable copper (EDTA-Cu) is increased to above 30 mg/kg (Purves, 1972) and that in the study by Batey et al, 12 kg/ha of slurry copper increased soil EDTA-Cu by 5.2 mg/kg in a single growing season.

Since zinc is added to high copper pig rations at a level of approximately 150 mg/kg D.M. and is excreted mainly in the faeces (Engel, Miller and Price, 1966) appreciable quantities of this element may be added to the soil along with copper in pig slurry. There is, however, no information available regarding soil contamination with zinc from this source.

Sewage sludges bear some similarities to pig slurries in that they have a certain fertiliser value, containing nitrogen, phosphorus and organic matter (Bunting, 1963) and also high concentrations of a number of trace elements (Le Riche, 1968; Berrow and Webber, 1972). There is also a considerable pressure to dispose of sewage sludges on agricultural land. McCalla, Peterson and Lue-Hing (1977) have reported concentrations of up to 4060 mg Cu/kg D.M. and 14,900 mg Zn/kg D.M. in some sewage sludges, while Berrow and Webber (1972) have shown that much of the zinc present may be in a readily soluble form. Le Riche (1968) found that heavy applications of sewage sludge increased acetic acid extractable copper levels in the topsoil from 7.0 to 24 mg/kg and zinc from 45 to 430 mg/kg. Leek plants grown on sludge treated soils contained significantly larger concentrations of copper and zinc than plants grown on untreated soils. Treatment of the plots with sludge was terminated and 6 years later it was observed that the level of extractable copper and zinc in the sludge treated soils had not changed appreciably. In studies with coastal bermuda grass and rye, King and Norris (1972 a,b) reported that crop removal of copper and zinc from sludge treated soil over a two year period was 0.3 to 0.9% of the copper and 1 to 1.3% of the zinc added in the sludge. Two years after the sludge was applied, 81% of the copper and 56% of the zinc was recovered in the sludge crust on the soil surface.

Purves (1972) has reported that the levels of copper and zinc were not substantially reduced on leaching columns of two heavily contaminated sludge treated soils with distilled water equivalent in volume to 40 metres rainfall. It would therefore appear that copper and zinc are strongly retained in soils treated with sewage sludge, suggesting that continuous addition of slurry copper and zinc to soils may result in an accumulation of these elements in the topsoil.

In general, the physical and chemical characteristics of a soil and the form in which heavy metals are added to the soil have an important influence on the distribution and fate of these elements in the soil-plant-animal system (Allaway, 1968). A major concern is that of the effect of the heavy metals, copper and zinc, on soil quality as measured by microbiological processes and chemical changes in the soil, by soil productivity and the nutritional consequences in livestock or man consuming crops grown on contaminated soils. The evaluation of potential hazards following contamination of soils requires a knowledge of the conditions under which these elements are retained in the soils. Since the factors governing the retention of elements such as copper and zinc may also influence availability of the elements to plants, the chemistry of copper and zinc in soils will be reviewed in the following section.

1.08. The chemistry of copper and zinc in soils.

According to Hodgson (1963), there are five basic ways in which trace elements may be retained in soils:

(i) they may be associated with soil surfaces either organic or inorganic;

(ii) they may become occluded during the development of new solid phases in which they are not principal constituents;

(iii) they can precipitate with other soil components forming a new phase;

(iv) they can occupy sites in soil minerals either as an original constituent or by entering the crystal lattice by solid state diffusion;

(v) they may become incorporated in biological systems and their residues in soils.

Unfortunately, the distinction between these forms is not always clear cut, the demarcation between surface adsorption and precipitation reactions, for example, being intrinsically diffuse.

Copper and zinc are also present in the liquid phase in soils as non-adsorbed ions, ions adsorbed on suspended colloids and ions complexed by soluble organic matter (Viets, 1962). In the solutions extracted from the surface horizons of soils so far studied, over 90% of the copper and 50% of the zinc have been found to be in a complexed form with total copper and zinc concentrations ranging from 0.01 to 0.06 and 0.03 to 3 μM respectively (Hodgson, Geering and Norvell, 1965; Hodgson, Lindsay and Trierweiller, 1966; McLaren and Crawford, 1973a). These complexes are anionic and are believed to be organic compounds since the degree of complexing has been found to be closely correlated with the organic matter content of the soil solutions (Hodgson et al, 1966).

The solid phase of soils contains relatively large quantities of trace elements in comparison with the soil solution. Vinogradov (1959) has estimated that the average total copper and zinc concentrations in soils are 10 and 50 mg/kg respectively. These metal cations may be bound to soil solids by a combination of forces ranging from weak electrostatic to covalent, with corresponding increases in bonding energy (Keeney and Wildung, 1977).

The more predominant reaction of heavy metals with soils and clay minerals would appear to involve surface adsorption. Copper may be adsorbed by soil colloids in amounts in excess of their conventional exchange capacities, assuming adsorption as the Cu^{2+} ion (Bower and Truog, 1940; DeMumbrum and Jackson, 1956; Bingham, Page and Sims, 1964). This adsorption may take place in the presence of amounts of calcium and other nutrient cations large enough to prevent adsorption on normal cation exchange sites. This phenomenon which occurs with copper, zinc and other heavy metal cations has been termed specific sorption and is exhibited by the clay minerals (Bingham et al, 1964; Tiller, 1968), organic matter (DeMumbrum and Jackson, 1956) and hydrous oxides of iron and manganese (McKenzie, 1967; Grimme, 1968). It has been suggested that the specifically adsorbed forms of copper may be in equilibrium with the very small amounts of solution and exchangeable copper in soils (McLaren and Crawford, 1973a). Furthermore, McLaren and Crawford (1973b) have shown that specific adsorption of copper depends mainly on the organic matter and free manganese oxide contents of soil and that clay minerals and free iron oxides are unlikely to be of major importance in retention of copper in soils due to the weak specific adsorption exhibited.

An important implication of basic studies on metal binding by soils is that, as the proportion of the soil's maximum adsorption capacity filled by a metal increases, the relative strength of binding decreases and hence, the relative availability of the metal to plants should increase. Shuman (1975) found that zinc adsorption at low and high solution zinc concentrations had markedly different binding energies and Bondetti and Sweeton (1973) found that the apparent stability constant of copper with soil organic matter fell by a factor of 10^2 to 10^3 as percent saturation with copper was increased from 5 to 50%.

An important factor in the reaction of metals with soils is a slow process of conversion of bound metals available to plants into forms which are no longer available. This process has been referred to as 'reversion' by Leeper (1972). Boawn (1974) found that extractability of zinc (22 kg/ha), added to soil as the sulphate, decreased slowly and reached a constant level after four years. Similarly Follett and Lindsay (1971) found reversion of Cu, Zn, Mn and Fe when added as soluble salts or chelates to a number of soils varying in texture and pH; in a period of 14 weeks extractability fell by more than 25%. However, it would appear that reversion may not always occur since Le Riche (1968) has reported that the extractability of copper and zinc in sewage sludge treated soils did not show any decrease from initial levels after a period of six years.

It is not yet known with any certainty which forms of copper are present in copper-rich pig slurry. If as suggested by Robel and Ross (1975) this element is present as the sulphide, it seems likely that this insoluble copper compound would be rapidly oxidised to the soluble sulphate in the moist aerobic conditions after spreading on land. In this situation it is to be expected that the slurry copper would enter into the equilibrium between soil solution and adsorption sites on soil minerals and soil or slurry organic matter. Alternatively, copper and zinc may be bound to the organic matter in slurry. It is not possible to predict which soil fraction these elements would enter on decomposition of the slurry organic matter by soil microorganisms; the elements might possibly be released into the soil solution or retained adsorbed on surfaces of materials that would eventually become humus.

While ion-exchange, absorption and precipitation phenomena all undoubtedly influence retention of trace elements in surface soils, differences exist in the relative importance, and rates, of the mechanisms involved depending on the metal, soil properties and environmental conditions. This is an extremely complex subject and the current literature is difficult to evaluate with respect to the relatively high concentrations of metals such as copper and zinc when added to soils in materials such as pig slurry or sewage sludge.

1.09. Factors affecting the availability of copper and zinc to plants.

For at least a century, soil has been extracted with water, salt solutions, acids and bases in attempts to relate the extractable quantities of trace elements in soils to the concentrations found in plants growing on the soils. The amounts extracted could vary from virtually none for elements such as copper and zinc when water was used, to almost the total amount in the soil when hot acids were used.

Viets (1962) has suggested that the behaviour of the trace elements in soils and their availability to plants might best be viewed in terms of a series of pools, each pool being the amount of an element in a given state that could be estimated by extraction or isotopic dilution techniques. Furthermore, each pool would have the attributes of concentration, size and equilibrium with other pools of that element. In this model, increases or decreases in availability are represented by shifts in the element from the individual forms in one pool to the forms in another. Each form is considered to be in equilibrium directly or indirectly with that in the soil solution from which plant uptake occurs. Since the availability of trace elements in soils is a function of their partition among the different forms, any factor which alters the equilibrium between these forms may have the potential to influence plant uptake.

It is commonly found that the availability of metals decreases or sorption increases with an increase in soil pH. Keeney and Wildung (1977) have attributed this to precipitation of manganese and other heavy metal oxides in soils, to secondary effects on the concentration of carbonate and phosphate in soil solution and to the rate and extent of sorption and desorption of metals by the soil hydrous oxides and organic matter. However, Hodgson (1963) has pointed out that in the case of copper and zinc, the amounts of these elements removed from the soil by chemical extraction vary more with soil pH than the amounts removed by plants. Furthermore, (MacLean, 1974) has found that while phosphorus additions to soils increase the level of extractable zinc, plant uptake of added zinc in the soils was decreased. The application of lime to the soil which in addition to increasing soil pH, introduces calcium and carbonate into the soil system, has been shown by Reith and Mitchell (1964) to consistently reduce plant uptake by grasses and clovers of naturally occurring copper and zinc but not of copper added to the soil.

In addition to its importance in the retention of copper, zinc and other trace elements in soils, soil organic matter may profoundly affect availability of the trace elements to plants. Three principal categories of soil organic matter have been proposed (Hodgson, 1963) although the complexity of the soil system results in considerable overlap between categories. These include (i) high molecular weight humic substances which have a high affinity for metals but are largely insoluble in soil thus immobilising metal cations associated with them (ii) low molecular weight acids and bases derived largely from microbial cells and which demonstrate relatively high solubility in association with metals and (iii) soluble ligands which are precipitated on reaction with metals. The influence of organic materials from categories (i) and (ii) on availability of copper is illustrated by the finding of Ennis and Brogan (1961) that copper added to a peat soil at a rate of 26 mg Cu/kg soil was bound so strongly as to be unavailable to oats. The addition of organic supplements to soils may increase the amounts of an element which are chemically extractable through the formation of soluble complexes. Miller and Ohlrogge (1958) have however observed that water extracts of manure reduced zinc uptake by plants despite the fact that these residues solubilised zinc in the soil.

Jenne (1968) has hypothesised that the influence of organic matter on trace element availability may be indirect in that this material may periodically give rise to a reducing environment with a lowering of pH and oxidation potential, thus affecting adsorption and the status of iron and manganese oxides. This hypothesis at least partially explains the commonly observed finding that on water-logging, soils containing sufficient organic matter to effect a decrease in oxidation potential, the availability of many metals including copper and zinc is increased (Kee and Bloomfield, 1962).

The possibility therefore exists that the organic matter already present in soil or added to the soil in pig slurry might, on the one hand reduce slurry copper and zinc availability, as a result of immobilisation or on the other hand increase availability through effects on soil pH and oxidation potential.

The available evidence supports the concept that ion uptake is related to ionic activities or the relative partial molar free energies of ions at the root surface (Baker, 1971, 1973). The activity or effective concentration at the root surface should be 0.02 to 0.04 mg/l for most crops and levels of 0.1 to 0.3 mg/l have been found toxic to crop plants (Hewitt, 1952; Smith and Specht, 1953; Struckmeyer et al, 1969). However, factors other than the chemistry of trace elements in soils may influence the uptake of these elements, and their concentration in plants. Metals may be actively absorbed, absorbed by passive diffusion due to ion activity gradients or inadvertently accumulated by an active mechanism for macro-nutrient cation absorption (Foy, Chaney and White, 1978). Competition among metals for chelation (Norvell, 1972; Lindsay,

1974) and hence movement to the root (Moore, 1974) have also been reported.

Translocation of metals from roots to tops remains poorly characterised. However, it now appears that copper translocation involves a specific chelator (Tiffin, 1972, 1977), which may possibly be involved in regulating movement of copper from the root. Jarvis, Jones and Hopper (1976) have reported that the roots of some crops eg. lettuce, release much more of their adsorbed cadmium for translocation to tops than other crops including the grasses. The copper content of ryegrass shoots was increased 4-fold, while that of the roots was increased 200-fold when the concentration of copper in the solution culture medium was increased from 0.16 to 157 μM (Jarvis and Jones, 1979). These workers also reported that although total copper uptakes by ryegrass and clover were similar when grown in flowing culture medium, white clover shoots contained 29 mg Cu/kg DM while those of ryegrass contained only 17 mg/kg DM. These differences in copper content of the shoots reflected the greater growth of clover roots and slower growth of clover shoots compared to ryegrass. Such species differences in translocation of trace elements from roots to shoots may well be of importance with regard to potential toxicity hazards to animals and man when soils are contaminated with heavy metals.

With regard to heavy metal contamination of agricultural soils Purves (1977) has reported that reduction in yield may occur in clovers at levels of EDTA-extractable copper in soils greater than 30 mg/kg while oats are unlikely to be affected below 100 mg/kg EDTA-Cu and in the case of zinc adverse effects on growth of oats and clovers are unlikely to occur at soil levels of acetic acid-extractable zinc below 200 mg/kg.

1.10. The decomposition of organic matter in soils contaminated with heavy metals.

Soil is the domain of myriads of microorganisms and small animals which are intimately associated with the soil organic fraction. This fraction represents a carbon, energy and nutrient source for most forms (Martin and Focht, 1977).

It has been observed that the ease of decomposition of different microbial and plant polysaccharides by soil microorganisms may vary considerably (Corpe, 1960; Martin and Richards, 1963). These polysaccharides have been shown to exist as metal salts or complexes (Mortensen, 1963). In a study of rates of decomposition in soil of prepared Cu, Zn, Fe and Al complexes of a number of polysaccharides, Martin, Ervin and Shepherd (1966) found that copper and zinc severely reduced the rate of decomposition of some complexes but had little effect on others. Copper apparently reduced degradation of the polysaccharides to the greatest degree. These workers concluded that salt or complex formation of organic materials with metal cations may markedly influence the rate of decomposition of these materials in soils and that specific decomposition rate effects may depend on the metal cation and the characteristics of the organic material. The implication of these findings is that heavy metals may exert an influence on the accumulation of organic matter in soils.

The mineralisation of nitrogen in soil has been reported to have been adversely affected by copper concentrations greater than 50 mg Cu/kg soil (Tyler, 1975). In contrast, Cornfield, Beckett and Davis (1976) found no evidence that copper at a level of 123 mg/kg in soil had reduced nitrogen mineralisation in soil or overall soil bacterial activity as assessed by carbon dioxide evolution. Cornfield et al suggested that the explanation for this discrepancy may lie in the fact that nitrogen mineralising bacteria are more susceptible to heavy metal toxicity than are carbon mineralisers. It is interesting to note that the soil used by Tyler (1975) had a very low pH, a condition under which heavy metals added to the soil may well have been more toxic.

Copper and zinc have been reported to inhibit bacterial activity in the determination of Biochemical Oxygen Demand (B.O.D.), a procedure designed to simulate the natural processes of purification of organic matter by oxidation in rivers. A Joint ABCM-SAC Committee on Methods for Analysis of Trade Effluents (J.C.M.A.T.E., 1957) have reviewed a number of publications regarding the determination of B.O.D. and have indicated that copper and zinc concentrations of 1 and 5 mg/l respectively in undiluted sewage sludge may inhibit oxidation by bacteria, while copper concentrations as low as 0.01 mg/l may be inhibitory in sewage sludge which has been diluted 100 times. Although it is not clear why copper should be inhibitory at a lower concentration on dilution of the sewage sludge suspension, it might be suggested that it is the concentration of dissolved copper and not the total copper concentration in the suspension which is of importance. It is already known that the fungicidal activity of copper is negligible when insoluble copper compounds are formed (Biedermann and Müller, 1951) and that the toxicity of high concentrations of dissolved copper to fish may be reduced by humic substances which remove copper from solution by complexation (Department of the Environment, 1971). An increase in dissolved copper concentration on dilution of sewage sludges for BOD determination may possibly arise from the use of a weak solution of ferric and calcium chloride as diluent (J.C.M.A.T.E., 1957), the Fe^{3+} and Ca^{2+} displacing copper from adsorption sites on the organic matter.

Since the slurry produced by copper supplemented pigs may contain high concentrations of copper, the possibility exists that the rate of decomposition of this material in soil or in biological treatment processes may be affected by the presence of this element. If, as suggested, it is the concentration of dissolved copper which adversely affects microbial activity, the decomposition of copper-rich slurry in soil would depend on the concentration of dissolved copper in the liquid phase of the slurry and the fate of this copper in soils.

Robinson, Draper and Gelman (1971) have reported that the rate of decomposition of organic matter was reduced in pig urine containing various concentrations of added copper and inoculated with bacteria. The lowest copper concentration, 50 mg/l, investigated by these workers was, however, very much greater than the concentrations reported to adversely affect B.O.D. determinations.

Although McGill, Jackson and Swinburn (1975) have reported concentrations of dissolved copper ranging from 0.4 to over 6 mg/l in pig slurry, the effect of this copper on decomposition of slurry solids in soil or biological treatment systems has not been investigated.

1.11. Copper poisoning in farm livestock.

Chronic copper poisoning may occur in animals under natural grazing conditions, as a consequence of excessive consumption of copper-containing mineral mixtures or through contamination of feeds with copper compounds from horticultural or industrial sources (Underwood, 1971). The effect of high concentrations of copper in the rations for pigs and the tolerance of this species to high dietary intakes of the element have been reviewed in section 1.05 of this chapter.

In all animals the continued ingestion of copper in excess of requirements leads to some accumulation in the tissues. Adult birds, like pigs, appear to be able to tolerate the accumulation of large amounts of copper in the liver without the occurrence of haemolytic jaundice or adverse effects on growth (Wiederanders, 1968). In ruminants, however, copper poisoning is an important nutritional problem.

Todd (1969) has drawn attention to the fact that the clinical picture in chronic copper poisoning is characteristic and quite different from acute poisoning where a single large dose of copper is thought to act as a corrosive problem causing enteritis, scouring, severe abdominal pain and perhaps death. Chronic copper poisoning on the other hand has been divided into two distinct phases:

(i) A period of passive accumulation of copper in the tissues, primarily the liver. This period of accumulation may vary from a few weeks to more than a year, during which time no symptoms of toxicity are exhibited.

(ii) The so called toxic phase which is an acute illness, often referred to as the haemolytic crisis. This phase progresses rapidly and usually ends in death in 2 to 4 days.

Sheep, the most susceptible species (Underwood, 1971) may be affected at any age although most reported cases have been in mature animals (Ogilvie, 1954; Clegg, 1956; Pearson, 1956; Pryor, 1959; Gracey and Todd, 1960). Young calves appear to be almost as susceptible to chronic copper poisoning as sheep, outbreaks due to excessive copper intakes having been reported on a number of farms (O'Moore, 1956; Shand and Lewis, 1957). Although older calves appear to be more tolerant, copper poisoning has occurred in yearling animals grazing pasture sprayed with copper sulphate (5.6 kg Cu/ha) as a molluscicide (Gracey and Todd, 1960). Mature cattle seem to tolerate high intakes of copper for extended periods. Cunningham (1946) reported no ill effects in a cow fed 5 g copper sulphate for 9 months, a level which would increase the dietary copper concentration by approximately 100 mg Cu/kg D.M.

The requirements of cattle and sheep for copper have been estimated to be 10 and 5 mg/kg respectively in the ration D.M. (A.R.C., 1965). Neathery and Miller (1976), in a review of maximum safe levels of trace elements in rations for farm livestock have reported that cattle may tolerate up to 100 mg Cu/kg in their rations without adverse effect. In contrast, copper poisoning has been reported in sheep fed rations containing 15 mg Cu/kg D.M. (Suttle, 1968; Buck, 1970), illustrating the narrow margin between sufficiency and toxic excess for this species. Thus, chronic copper poisoning in ruminants may be regarded as a disorder almost entirely confined to sheep, with young, milk-fed calves also at risk.

The normal concentration of copper in the liver of sheep rarely exceeds 50 mg/kg fresh tissue (250 mg/kg D.M.), whereas excessive copper storage may result in copper concentrations in excess of 1000 mg/kg fresh tissue (Todd, 1969). During this period of accumulation the levels of copper in blood and other tissues remain within the normal range (Todd and Thompson, 1963; McCosker, 1968). Increases in the activity of the enzyme glutamate-oxaloacetate transaminase in serum (S.G.T.O.) have been shown to occur up to 8 weeks before the haemolytic crisis occurs, indicating liver damage (Todd and Thompson, 1963; Ross, 1964).

In a recent review, Bremner (1974) has pointed out that, although the onset of the haemolytic crisis in sheep has been known for many years to be precipitated by a number of stress factors, including starvation, the underlying mechanism has still not been established. The haemolytic crisis involves a catastrophic release of copper from the liver into the blood stream, blood concentrations of the element rising in some cases to twenty times the normal level, resulting in haemolysis 24 to 48 hours later (Todd, 1969). This sequence of events and the biochemical changes taking place during the haemolytic crisis are quantitatively similar in both sheep and calves (Todd, Gracey and Thompson, 1962; Todd and Thompson, 1963, 1965). In sheep, death usually occurs within about 2 days of onset of the crisis, although a proportion of individuals of some breeds, such as Merinos, may survive (Marston, 1950).

It would appear from reported outbreaks of copper poisoning in sheep (Bracewell, 1958; Senior, 1959; Gracey and Todd, 1960) that only a proportion of a flock are likely to undergo a haemolytic crisis, suggesting that there may be variation between individual sheep in their ability to retain copper in the liver. Todd (1969) has reported that liver copper concentrations ranged from 100 to over 500 mg/kg fresh tissue in a group of sheep fed the same ration for a period of 10 months. Furthermore, evidence has been obtained demonstrating that the susceptibility of sheep to copper poisoning following therapeutic use of copper (Wiener and McLeod, 1970) varies among breeds and that concentrations of copper in blood (Wiener, Hall, Hayter, Field and Suttle, 1974) and in liver (Wiener, Herbert and Field, 1976) may be under a measure of genetic control. Recently, deaths due to copper poisoning have been reported in a small flock of Orkney sheep from the island of North Ronaldsay when taken to grazing on a farm in southern Scotland; other breeds of sheep rapidly become copper deficient on this farm and investigations have suggested that the 'North Ronaldsay' breed have a greater ability to absorb dietary copper than the other breeds studied (Wiener, Field and Smith, 1977).

The metabolism of copper in the ruminant represents a very complex situation affected not only by the intake of copper itself, but by other factors in the diet such as molybdenum and sulphur (Dick, 1954; Suttle, 1974; Suttle and McLauchlan, 1976), zinc (Bremner and Marshall, 1974) and iron (Campbell, Coup, Bishop and Wright, 1974). Attempts have been made to prevent the occurrence of copper poisoning in sheep by dietary supplementation with molybdenum and sulphate (Ross, 1966, Hogan, Money and Blayney, 1968) but, despite the frequent success of this measure, there has been reluctance to adopt it in commercial practice for fear of inducing a copper deficiency state (Bremner, Young and Mills, 1976).

Zinc supplementation of pig rations has been used successfully to reduce the toxicity of high dietary levels of copper to the pig (Suttle and Mills, 1966). Increase in the concentration of zinc in the liver of sheep has been shown to result in a redistribution of hepatic copper, with an increase in the amount bound to metallothionein, which is thought to be involved in the storage and detoxification of copper (Bremner and Marshall, 1974). In view of the fact that ruminants may tolerate up to 500 mg Zn/kg in the ration (Bremner, 1974), preliminary studies were carried out by Mills (1974) which demonstrated that increasing the dietary zinc concentration to 100 mg/kg resulted in a reduction in the liver copper content of sheep. Bremner, Young and Mills (1976) in a further investigation of this zinc effect have reported that, in sheep fed rations containing 29 mg Cu/kg, a substantial protection against copper poisoning was obtained by increasing dietary zinc concentration to 220 mg/kg, while 420 mg Zn/kg diet was even more effective. These levels of zinc had no adverse effect on growth rate although complete protection against liver damage due to copper was not obtained.

Symptoms resembling those of copper deficiency have been observed (Coup and Campbell, 1964) in cattle grazing pasture irrigated with water containing high concentrations of iron. More recent investigations (Campbell, Coup, Bishop and Wright, 1974) into the effect of iron on copper metabolism have shown that, in cattle, a daily dose of 7 mg Fe/kg live weight may significantly reduce storage of copper in the liver. In sheep, Abdellatif (1968) has recorded a lower rate of copper accumulation in the liver of sheep fed rations supplemented with iron salts compared to sheep fed unsupplemented rations. As yet, the mechanism for this interaction is not known and further research may be required to assess the potential of iron supplements in protecting sheep from copper poisoning arising from high dietary copper intakes.

From the literature reviewed, it would seem unwise to allow sheep access to grazing or rations containing copper in excess of 5 mg/kg D.M. as recommended for this species (A.R.C., 1965) unless factors which are known to reduce copper absorption or retention are naturally present or deliberately added to the diet.

The disposal of copper-rich pig slurry on pasture may constitute a potential toxicity hazard to sheep since this practice has been shown to increase herbage copper concentrations to well in excess of 5 mg/kg D.M. (Batey et al 1972; Lawrence and Gibbs, 1973; Kneale and Howell, 1974). Furthermore, inclusion of pig excreta in rations for ruminants (Bhattacharya and Taylor, 1975) may increase dietary copper levels. Since it has been reported (Field and Purves, 1964; Healy, 1973) that grazing livestock may ingest soil along with grass, the possibility exists that slurry solids on the soil surface after application may also find their way into the animals diet in this fashion.

Although the ingestion of slurry copper by sheep has been the subject of a number of studies, the evidence presented with regard to the risk of poisoning from this copper source has been conflicting. Thus Woodside (1973) concluded that slurry copper presents little risk of copper poisoning to grazing sheep, while Dalgarno and Mills (1975) have suggested that prolonged ingestion of slurry copper may result in accumulation of copper to high concentrations in the liver of sheep. The answer to this question regarding the potential toxicity of slurry copper to grazing livestock will, however, have to await further research.

CHAPTER 2.

INVESTIGATION OF THE EFFECT ON CONCENTRATION OF COPPER IN CLOVER AND RYEGRASS OF ADDITION OF COPPER-RICH PIG SLURRY AT VARIOUS LEVELS TO SOILS UNDER FIELD CONDITIONS.

2.01. INTRODUCTION

In view of the ability of ruminants to accumulate high concentrations of copper in the liver and the susceptibility of sheep in particular to copper toxicosis (Underwood, 1971), any action producing a marked increase in copper intake must be regarded as a potential hazard.

Increases in herbage copper content following repeated, heavy applications of copper-rich slurry to established grass/clover swards have been reported (Batey et al, 1972; Lawrence and Gibbs, 1973; Kneale and Howell, 1974). Little information is available however, with regard to the persistance of elevated herbage copper concentrations after slurry application has been discontinued. In addition, no attempt has been made to examine the effect of slurry copper on the concentration of this element in clovers relative to that of grasses. Beck (1962) has reported that the copper concentration in grasses is usually slightly greater than that in clovers when clovers contain less than 4 mg Cu/kg D.M.; at copper concentrations in clover greater than 4 mg Cu/kg D.M., grasses were found to contain considerably less copper than the clovers grown on the same soils. Furthermore, since the incorporation of inorganic copper salts into soils is known to produce a greater response, in terms of copper concentration in plant tissues, with clovers than with grasses (Purves, 1965; Reith and Mitchell, 1964), slurry copper might be expected to have a more pronounced effect on the copper content of herbage in situations where clovers occur extensively in the sward.

Quite apart from the differences in content between various plant species, the concentration of this element within any one species may, as reviewed earlier, vary markedly depending on the characteristics of the soil on which a crop is grown. It would therefore appear likely that the soil characteristics governing the availability of native copper to plants would also, to some extent at least, influence the availability of slurry copper and its uptake by plants when it is added to different soils.

In order to extend present information on the effects on copper content of herbage resulting from the disposal of copper-rich pig slurry on farm land, an experiment was designed with the following specific objectives:-

(i) To examine the effect on copper concentration in a mixed white clover/perennial ryegrass sward of a single addition to the soil of various amounts of slurry copper, slurry application rates being chosen to provide the major nutrient, nitrogen, at rates increasing from normal to excessive (MAFF, 1976) for grazing or silage production.

(ii) To compare the effect of slurry addition to two soils, differing with respect to level of chemically extractable copper, on the concentration of copper in mixed herbage grown on the soils.

(iii) To compare the concentration of copper attained by clover with that of ryegrass following slurry addition to the soil.

2.02. Site selection and preparation.

Samples of topsoil were taken by screw auger from a number of soil series within associations (The Soils of the Bush Estates, Midlothian, 1969) present on the East of Scotland College of Agriculture (E.S.C.A.) farms, Midlothian. The levels of extractable copper in these samples were determined using 0.02 M EDTA extractant (di-sodium salt, pH 4.2 in distilled water) as in the method of Henriksen and Jensen (1958). On the basis of differing soil copper extractabilities, two sites measuring 30 x 30 metres (m) were selected on uniform areas of established perennial ryegrass swards. Site A was located on the Darvel soil series, Darvel Association, a freely drained brown forest soil derived from fluvioglacial and lacustroglacial sands and gravels. Site B was located on the Winton soil series, Rowanhill Association, an imperfectly drained brown forest soil with gleyed B and C horizons. Further characteristics of these soils are given in Appendix I.

Each site was ploughed and rotavated to produce a seedbed suitable for establishment of a grass/clover sward. A total of 20 plots were marked out on each site, with 1 metre between plots.

2.03. Source, storage and composition of copper-rich pig slurry.

Copper-rich pig slurry was obtained from under-floor storage tanks in the pig fattening unit at Easter Howgate on the E.S.C.A. farms; the fattening rations used contained 200 mg Cu/kg D.M. Maximum storage time for slurry in these under-floor tanks was 4 weeks. Approximately 3000 litres (l) of slurry was transferred to a bitumen lined fibre-glass tank on each site in May 1972 and again in May 1973 and each tank tightly covered with polythene sheeting. The slurry consisted of urine and faeces with no wash-water added; the composition of this material used on each site in 1972 and 1973 is shown in Table 2.1.

TABLE 2.1.

Composition of slurry applied on sites A and B

Year	Site	Percentage of fresh slurry: Dry matter	N	P	K	Cu (mg/kg DM)
1972	A	9.0	0.75	0.23	0.30	725
	B	11.6	0.77	0.26	0.27	719
1973	A	13.7	0.48	0.20	0.53	536
	B	15.1	0.49	0.23	0.37	524

2.04. Treatments applied.

Four treatments were applied in 1972 and 1973, with each treatment replicated 5 times in a randomised block design on each site.

The treatments (T) in 1972 and 1973 were:

T1	Control; mineral fertiliser only 1972, 1973		
T2	25,000 l slurry/ha, 1972;	25,000 l slurry/ha, 1973	
T3	50,000 l " " ;	37,500 l " "	
T4	100,000 l " " ;	50,000 l " "	

Since Mc Allister (1963) has observed that, in terms of plant uptake, the efficiency of nitrogen is only 66% of that of ammonium sulphate, a mineral fertiliser (SAI No. 2, Scottish Agricultural Industries, Leith, Scotland) was applied to control plots to provide 66% of the N added at the lowest rate of slurry application. The rate of application of the mineral fertiliser would provide sufficient P and K for a single cut of silage (MAFF, 1973). Details regarding the amounts of N, P, K and copper added to the soils in each treatment are given in Table 2.2.

The slurry was agitated in the storage tanks prior to application to the plots to ensure even distribution of the solids and 4 x 250 ml samples were taken as slurry was drawn from the tanks for spreading. The slurry samples were stored for analysis at $-15^{\circ}C$ in sealed polythene containers.

Slurry with a dry matter content of 10% is an extremely viscous liquid. In order to achieve even application to the

TABLE 2.2.

Amounts of N, P, K and Cu added to the soil in slurry or mineral fertiliser

	Treatment	Rate of addition (kg/ha)			
		N	P	K	Cu
SITE A 1972	*T1	125	28	51	<0.01
	T2	188	58	75	1.67
	T3	375	115	150	3.34
	T4	750	230	300	6.69
SITE B 1972	*T1	125	28	51	<0.01
	T2	193	65	68	2.15
	T3	385	130	135	4.31
	T4	770	260	270	8.62
SITE A 1973	*T1	82	18	34	<0.01
	T2	120	50	133	1.97
	T3	180	75	199	2.95
	T4	240	100	265	3.94
SITE B 1973	*T1	82	18	34	<0.01
	T2	123	58	93	2.07
	T3	180	86	139	3.10
	T4	245	115	185	4.13

*S.A.I. Mineral fertiliser No. 2; 22% N, 11% P_2O_5, 11% K_2O: Scottish Agricultural Industries, Leith, Scotland.

surface of treated plots using a watering can with a plastic deflector plate on the spout, the slurry was diluted with an equal volume of water before spreading, thus reducing its D.M. content to approximately 5% in 1972 and 7.5% in 1973. The mineral fertiliser was dissolved in water to give a liquid application rate of 50,000 l/ha equivalent to that of slurry + water in treatment T1.

Mineral fertiliser and slurry at the rates shown above were applied to the experimental plots in May 1972 and the surface of each plot raked lightly when it had dried out. A mixture of British Certified S23 perennial ryegrass and S100 white clover (2:1 by weight respectively) was then sown on the site at a rate of 10 kg/ha, raked in and the soil surface consolidated by rolling. The plots received no further application of slurry or fertiliser until July, 1973. Before this second application the established sward was cut back close to ground level in order to minimise foliar contamination of the herbage.

A representative sample of herbage was cut, at a height approximately 2 cm above ground level using stainless steel shears, 10 weeks after sowing in 1972, by which time the sward had reached a height of 15 cm (grazing stage). The sample from each plot, approximately 200 g fresh weight, comprised of 10 sub-samples taken at random from within a plot. The crop was then cut, removed and the regrowth sampled at the grazing stage, 4 weeks later.

Following slurry application in 1973, samples of the mixed herbage were taken at grazing stage and later when the grass reached ear-emergence stage. Clover was separated from grass by hand in sub-samples from control and treatment T4 plots at grazing stage for determination of the copper content of each species separately. The crop was again cut, removed and without further slurry application, the regrowth was sampled at grazing stage.

Soil samples were taken from all plots by screw auger 14 weeks after slurry application in 1972 and 7 weeks after application in 1973; sampling depth was 0 to 15 cm and each sample comprised of 8 sub-samples from points taken at random within a plot.

2.05. Chemical and other analyses.

Copper in soil was extracted with 0.02 M ethylenediamine-tetra acetic acid (EDTA) or 1 N hydrochloric acid in distilled water. Details of extraction procedures employed are given in Appendix II. A 10 ml aliquot of copper sulphate solution containing 2.5 mg Cu/l was added to a number of soil samples from sites A and B before addition of extractant, in order to assess recovery from slurry treated or untreated soils.

Details of the procedures used in the determination of total N, P and K in pig slurry are given in Appendix III and the determination of Cu concentrations in herbage and slurry solids is described in Appendix IV.

An analysis of variance was carried out on the data for copper concentration in the mixed herbage and on the data for EDTA-extractable copper in soils. The mean concentrations of copper in ryegrass and clover were compared using Students t-test.

2.06. Results.

The recovery of copper, added as copper sulphate to 10 soil samples from sites A and B immediately before extraction, was in excess of 97.5% whether the soils contained slurry or not.

The mean concentrations of EDTA-extractable copper in soils from each site after fertiliser or slurry treatment and the percent extractability of the slurry copper added to the soil are shown in Table 2.3. The imperfectly draining clay-loam soil of site B had a higher concentration of EDTA-Cu than the freely draining sandy soil of site A as shown by the respective mean values of 6.2 and 2.2 mg Cu/kg soil in 1972. The same pattern of extraction was found with 1 N HCl although the amounts of copper extracted from both soils were greater with the strong acid than with EDTA.

Incorporation of slurry into the seedbed and surface application to the established sward increased EDTA-Cu and HCl-Cu in soils of both sites to levels significantly greater than those of the control plots. The increases above control levels in 1972 were, however, small; the extractable copper levels in soil receiving slurry at the lowest rate of application did not

TABLE 2.3.

Effect of slurry copper application to soil on mean EDTA-extractable (EDTA-Cu) and HCl-extractable (HCl-Cu) copper in topsoil

Copper applied (kg Cu/ha)	EDTA-Cu (mg Cu/kg soil)	HCl-Cu (mg Cu/kg soil)	Extractability of total applied copper (%) EDTA	HCl
Site A, 1972				
T1, NIL	2.2	4.1	-	-
T2, 1.67	2.4	4.3	23	25
T3, 3.34	2.9	4.8	42	47
T4, 6.69	3.9	5.8	51	53
LSD ($P<0.05$)	0.56	0.51		
Site B, 1972				
T1, NIL	6.2	8.8	-	-
T2, 2.15	6.4	9.0	13	15
T3, 4.31	6.7	9.4	22	24
T4, 8.62	7.2	9.9	22	23
LSD ($P<0.05$)	0.38	0.39		
Site A, 1973				
T1, NIL	2.0	4.0	-	-
T2, 1.97	2.5	4.5	27	28
T3, 2.95	3.0	5.0	32	34
T4, 3.94	4.0	6.2	40	44
LSD ($P<0.05$)	0.28	0.36		
Site B, 1973				
T1, NIL	6.1	8.9	-	-
T2, 2.07	6.5	9.3	16	19
T3, 3.10	6.8	9.6	18	20
T4, 4.13	7.4	10.1	20	20
LSD ($P<0.05$)	0.34	0.32		

LSD = Least Significant Difference between means.

Extractability of applied Cu calculated using bulk density values, APPENDIX I.

differ significantly from the control levels on each site. The maximum concentration of EDTA-Cu and HCl-Cu was found in soil at the highest rates of slurry application, which added 6.69 and 8.62 kg Cu/ha in 1972 with a further 3.94 and 4.13 kg Cu/ha in 1973 to sites A and B respectively. In all treatments the concentration of EDTA-Cu and HCl-Cu was greater in the imperfectly drained, clay-loam soil of the Winton series than in the freely draining, light sandy soil of the Darvel series.

The estimated recovery of slurry copper from soil by extraction with EDTA and HCl was low on both soils, the maximum extractability with EDTA being 51% for soil of the Darvel series, site A and 22% for soil of the Winton series, site B 14 weeks after the first application in 1972. The percent extractability of the added copper was greater in treatment T3 than in T2 on both sites in the first and second season; extractability showed little further increase, however, when slurry copper was applied at the highest rate in treatment T4. The extractability of the total amount of copper added in 1972 and 1973 was found to be lower than that of the copper added in 1972. Furthermore, both extractants used removed a greater percentage of the added copper from soil of the Darvel series than from soil of the Winton series.

Although the strongly acidic extractant, 1N HCl, removed greater amounts of copper from the soils than did the complexing agent, EDTA, as shown by the values for plots receiving mineral fertiliser only in treatment T1, the extractability of slurry copper in soil was found to be similar for the two extractants.

The mean pH values for soils receiving each treatment on site A and B are shown in Table 2.4. The single application of slurry at rates varying from 25,000 to 100,000 l/ha in 1972 and 25,000 to 50,000 l/ha in 1973 had no significant effect on soil pH.

TABLE 2.4.

Effect of slurry application on pH of topsoil (0-15 cm)

	Site	Treatment				Variance Ratio (P<0.05)
		T1	T2	T3	T4	
1972	A	6.3	6.4	6.4	6.5	N.S.
	B	6.2	6.3	6.4	6.4	N.S.
1973	A	6.4	6.3	6.4	6.3	N.S.
	B	6.3	6.2	6.3	6.4	N.S.

N.S. = Treatment means not significantly different

The mean concentrations of copper in mixed herbage following the application of mineral fertiliser or pig slurry to the soil are shown in Table 2.5. Slurry applied at the rate of 100,000 l/ha to the soil in 1972 resulted in a significant increase in herbage copper content at grazing stage (15 cm in height) on the sandy soil of site A only; the herbage copper concentrations were 4.3 and 5.6 mg Cu/kg D.M. for the mineral fertiliser and slurry treatment respectively. Herbage copper concentrations at the two lower rates of application, T2 and T3, on site A and at all rates on site B did not differ

TABLE 2.5.

Effect of slurry application to the seed bed (1972) and to the established grass/clover sward on copper content of the mixed herbage

Site and Year	Copper applied (kg/ha)	Mean copper concentration (mg/kg D.M.)		
Stage of growth		grazing	ear-emergence	grazing
A 1972	T1, NIL	4.3	ND	6.1
	T2, 1.67	4.5	ND	6.3
	T3, 3.34	4.7	ND	6.5
	T4, 6.69	5.6	ND	6.5
LSD (P<0.05)		0.74		N.S.
B 1972	T1, NIL	5.5	ND	7.0
	T2, 2.15	5.4	ND	6.9
	T3, 4.31	5.8	ND	7.5
	T4, 8.62	6.1	ND	8.7
LSD (P<0.05)		N.S.		0.53
A 1973	T1, NIL	4.9	5.0	4.8
	T2, 1.97	6.0	5.1	5.4
	T3, 2.95	9.2	7.1	6.9
	T4, 3.94	12.4	6.9	7.2
LSD (P<0.05)		1.06	0.52	0.69
B 1973	T1, NIL	10.1	7.9	8.5
	T2, 2.07	10.3	8.1	8.3
	T3, 3.10	12.2	9.8	9.3
	T4, 4.13	15.6	10.1	9.3
LSD (P<0.05)		0.91	0.88	0.64

ND = Not determined.

LSD = Least Significant Difference between treatment means.

N.S. = No significant difference between treatment means.

significantly from the control levels. Slurry applied at the rate of 100,000 l/ha resulted in the addition of 6.69 and 8.62 kg Cu/ha to the soils of sites A and B respectively, while the mineral fertiliser containing 4.8 mg Cu/kg added less than 0.01 mg Cu/ha to the soil.

The first crop having been harvested, no further slurry was applied to the soil in 1972. At grazing stage, the mean copper content of the regrowth on plots previously treated with slurry was found to be significantly greater than that of the control only at the highest level of slurry application on the imperfectly drained clay-loam soil on site B.

In the second year of the experiment, 1973, the ryegrass/clover sward was trimmed back to ground level before slurry was applied. Slurry application resulted in significant increases in the copper content of the regrowth sampled at the grazing stage except at the lowest slurry application rate on site B. The maximum increases in herbage copper content were 6.5 and 5.6 mg/kg D.M. on the light, freely drained soil on site A and the heavy, imperfectly drained soil on site B respectively. These increases correspond to herbage copper concentrations of 12.4 and 15.6 mg Cu/kg D.M. respectively on sites A and B.

Herbage sampled four weeks later when grass in the mixed sward reached ear-emergence stage, showed a decline in copper content. However the mean copper concentrations in herbage from plots which had received 37,500 or 50,000 l slurry/ha remained significantly greater than those of herbage receiving the mineral fertiliser. After harvesting the crop, and without further slurry application, the regrowth in all slurry treatments on site A and in those on site B excepting the lowest application rate T2 showed higher copper concentrations than the sward receiving mineral fertiliser alone.

The copper content of herbage on site B was consistently greater than that on site A in all treatments and at the grazing and ear-emergence stages of growth.

The concentration of copper in ryegrass and clover at the grazing stage, 5 weeks after slurry application in 1973, is shown in Table 2.6. Copper concentration in clover was found to be greater ($P<0.001$) than in ryegrass in both treatments on each site.

TABLE 2.6.

Mean copper concentration in perennial ryegrass and white clover at grazing stage

Site	Slurry applied (kg/ha)	Copper concentration (mg/kg ± SE)		Clover:Grass (D.M.)
		Ryegrass	Clover	
A	NIL	4.7 ± 0.32	5.8 ± 0.09	1:4.6
	3.94	10.8 ± 0.63	19.1 ± 1.46	1:4.3
B	NIL	9.5 ± 0.39	12.1 ± 0.43	1:3.3
	4.13	13.4 ± 0.59	24.8 ± 1.25	1:4.3

2.07. Discussion.

The incorporation of copper-rich pig slurry into soil at rates supplying the major nutrients N, P and K at levels from normal to excessive in terms of requirement (MAFF, 1973) resulted in enhancement of EDTA-extractable and HCl-extractable copper in soil and of the copper content of ryegrass growing on the soil.

The EDTA-extractability of the slurry copper added to the soils showed considerable variation, ranging from 23 to 51% and 13 to 22% from lowest to highest application rates on sites A and B respectively, in the first year of the experiment. In contrast, Batey et al (1972) reported an EDTA-extractability of approximately 50% for slurry copper added in various amounts to soil. The strongly acidic extractant, 1 N HCl, was found to remove similar quantities of added slurry copper from the soil as did EDTA. Since the extractability of copper from slurry alone has been found to be 100% (this thesis, chapter 4), the variation in extractability in relation to the amount of copper added to the soil and the variation from year to year may, to a certain extent, be due to the sampling technique used. In support of this explanation, it was observed that slurry solids remained on the soil surface after spreading in the second year of the experiment and that the surface 2.5 cm layer of soil and slurry solids was often lost on withdrawal of samples from the soil using a screw auger. It would therefore appear likely that the extractability of slurry copper in soil has been seriously underestimated and it is suggested that, where slurry has been applied to soil the method of soil sampling should be chosen with a view to ensuring recovery of all surface soil and surface debris.

The highest mean concentrations of EDTA-extractable copper in the topsoil, which may only be representative of the soil below the surface 2.5 cm layer on slurry treated plots, were 4.0 and 7.4 mg Cu/kg soil for sites A and B respectively. Although a total of 10.63 kg/ha (site A) and 12.75 kg slurry copper/ha (site B) had been added to the soil in two slurry applications, these levels are considerably lower than the mean EDTA-Cu content, 11.5 mg Cu/kg reported by Purves (1970) for a number of rural soils in Scotland.

Purves (1977) has indicated that phytotoxicity, characterised by a chlorosis resembling that occurring in iron deficiency, is unlikely to occur in clovers or oats until soil EDTA-Cu levels exceed 30 mg/kg soil for the former and 100 mg/kg soil for the latter species. There would therefore appear to be very little likelihood of phytotoxicity occurring in clover/ryegrass swards as a result of single applications of up to 100,000 l slurry/ha.

The finding that slurry application did not affect soil pH is in agreement with the work reported by Batey et al (1972) and Mutlak (1974).

In the first year of the experiment, incorporation of the slurry copper into the seed bed did not significantly increase copper levels in the first crop of herbage at grazing stage 12 weeks after the slurry was applied on site B; on site A the increase in herbage copper content, 1.3 mg Cu/kg D.M., at the highest rate of applied slurry was small. In contrast, the same slurry applied to an established ryegrass sward on the same soils and at similar rates (this thesis, chapter 3) resulted in a three-fold increase in the copper content of the grass samples at grazing stage, 4 weeks after slurry application. Furthermore, smaller amounts of slurry copper when applied to the established grass/clover sward in the second year of the experiment resulted in marked increases in the copper content of the herbage at the same stage of growth as in the previous year. Batey et al (1972) have also reported increases of up to 28 mg Cu/kg D.M. in herbage within a period of 4 weeks following application of copper-rich pig slurry at rates and under conditions similar to those in this experiment.

These findings would suggest that slurry copper added to the soil may rapidly become unavailable to plants, despite the fact that up to 50% of the slurry may remain in the soil in a form extractable with EDTA. The decreased levels of copper in the regrowth at grazing stage and the observation (Reith and Mitchell, 1964) that the availability to plants of copper from inorganic copper salts added to soil decreases with time, would lend support to this hypothesis. From the results of this experiment it is not clear whether the lower copper content of the mixed herbage at ear-emergence stage compared to that at grazing stage is a consequence of decreasing availability of the slurry copper to the plants or an effect of increasing maturity of the herbage, mature herbage commonly containing lower concentrations of copper than herbage at grazing stage (Burridge, 1969).

The concentration of copper in clover is generally greater than that in grasses, except where the soil is severely deficient in this trace element (Mitchell, 1957; Beck, 1962). Furthermore, the addition of inorganic copper salts to the soil (Reith and Mitchell, 1964) or to solution culture medium (Jarvis and Jones, 1979) results in greater copper concentrations in the clovers than in grasses. Perennial ryegrass and white clover were found to follow this pattern when the source of copper was pig slurry. Addition of approximately 4 kg Cu/ha to the soil increased the concentration of copper in ryegrass from 4.7 to 10.8 mg/kg D.M. on the light, sandy soil of the Darvel series and from 9.5 to 13.4 mg/kg D.M. on the clay-loam soil of the Winton series. The corresponding increases in the copper concentration in white clover were from 5.8 to 19.1 mg Cu/kg D.M. and from 12.1 to 24.8 mg Cu/kg D.M. on these soils. In comparison, Mitchell (1957) has reported that the copper content of clovers ranged from 3.8 to 10.2 mg/kg D.M. and that of ryegrass from 2.4 to 4.3 mg/kg D.M. when grown on 15 different soils in N.E. Scotland.

Clover in the sward was found to constitute approximately 20% of the total dry matter content of the sward in the mineral fertiliser and slurry treatments. The proportion of clover in the sward was thus apparently unaffected by copper-rich pig slurry at rates of 100 and 50 thousand l/ha in two applications, one year apart. From a consideration of the soil factors known to affect copper availability to plants (reviewed, section 1.09) it would appear that the greatest concentrations of copper in pasture are likely to occur when copper-rich pig slurry is applied to heavy, poorly drained soils with low pH and where there is a high proportion of clover in the sward.

The situation is less clear, however, with regard to the disposal on pasture of copper-rich pig wastes which have undergone some form of biological treatment. Mutlak (1974) has reported that supernatant effluent liquor from an oxidation ditch of the type described by Robinson, Saxon and Baxter (1971), did not consistently increase the copper content of perennial ryegrass (P.R.G.) grown under greenhouse conditions. Increasing levels of effluent addition apparently resulted in decreases in uptake of copper by the crop as assessed from recovery in the crop, of copper applied to the soil. However, the concentration of copper in herbage from the control treatment was reported to be 23 mg Cu/kg D.M., an abnormally high copper level for herbage growing on uncontaminated soil. An explanation for the inconsistent effect of effluent copper on concentration of this element in the herbage may lie in the control of copper transport from roots to tops under conditions of high availability to the roots as described by Jarvis and Jones (1979).

From the results of this investigation, a number of points have emerged, which must be taken into consideration in assessing the toxicity hazard to livestock grazing pasture treated with copper-rich pig slurry. Within the limits of the experimental treatments, herbage copper concentration increased with increasing rate of slurry application and hence the risk of copper poisoning in susceptible species such as sheep is likely to be greater, the larger the amount of slurry copper applied to pasture in a single application. A high proportion of clover in the sward may add further to this risk. The characteristics of soils may also influence the copper content of the crop. While herbage grown on the poorly drained clay loam soil had a higher copper content than that grown on the freely drained, sandy soil, the availability to the crop of the slurry copper added to the soil apparently decreased rapidly in both soils within a matter of weeks following slurry application.

While this experiment has provided information on the short-term effects of disposal of copper-rich pig slurry on agricultural land, it gives little indication of the consequences of continued long-term disposal on the accumulation of copper in soils and the extent to which this may increase the concentration of copper in crops. These long-term aspects were therefore made the subject of further investigation.

CHAPTER 3.

INVESTIGATION OF THE ACCUMULATION OF COPPER IN SOIL AND THE COPPER, ZINC AND MANGANESE CONCENTRATIONS IN PERENNIAL RYEGRASS FOLLOWING REPEATED APPLICATIONS OF COPPER-RICH PIG SLURRY TO AN ESTABLISHED SWARD DURING TWO GROWING SEASONS.

3.01. Introduction

Hodgson (1963) has stated that "mobility of an element in soils is no more than a reflection of its solution concentration as it is affected by the movement of water through the profile" and that "any factor that affects the solubility of an element must in the same way affect its movement". In view of the extremely low concentrations of copper, 0.01 to 0.06 µM reported in soil solution (Hodgson et al, 1966) it is not surprising that copper in soils has been shown to move very little in the soil profile (Jones and Belling, 1967). The accumulation of high concentrations of copper and zinc (Le Riche, 1968) and zinc (Patterson, 1966) have been reported in soils which had received repeated dressings of sewage sludge. Le Riche (1968) found that heavy applications of sewage sludge increased the extractable levels of copper and zinc in the topsoil, and that these levels had not declined 6 years after sewage application had been terminated. The low mobility of zinc in soil contaminated with this element from an industrial source has been described by Crossman, 1974; in spite of soil cultivation enhanced soil zinc levels were only found down to a depth of 15 cm in the soil. Purves (1972) in a discussion of consequences of trace element contamination of soils has concluded that, with respect to copper and zinc, contamination of soils may be irreversible and has shown that the levels of these two elements were not substantially reduced after leaching columns of sewage sludge treated soils with a volume of distilled water equivalent to 40 m rainfall. There would therefore appear to be little doubt that, once added to soils, these elements are unlikely to be leached out under normal circumstances.

There appears to be virtually no published information regarding the accumulation of copper from pig slurry in soil. The results of the previous experiment (Chapter 2, this thesis) are in agreement with the findings of Batey et al (1972) indicating that, in the short-term at least, up to 50% of slurry copper added to the soil may be recovered by chemical extraction. In view of the irreversible nature of contamination of soils with copper and zinc and the possible deleterious effect on the plant animal system, it was decided to monitor the effects of moderate applications of copper-rich slurry on the concentrations of copper in soil and perennial ryegrass growing on the soil. Since on preliminary examination, pig slurry was found to contain iron, manganese and zinc of dietary origin, at concentrations in excess of 400 mg/kg D.M. the concentrations of these elements were also monitored in herbage.

In order to assess whether or not copper in herbage has been taken up from the soil or is simply present in slurry adhering to the aerial parts, herbage may be examined visually for surface contamination or washed before analysis; these methods are either not very reliable or are extremely time consuming. The extremely high iron concentrations observed in pig slurry (> 1000 mg Fe/kg D.M.) gave rise to the idea that herbage iron levels may possibly be useful in indicating whether or not herbage samples are surface contaminated with slurry, providing that iron uptake via the roots is not increased by slurry application to the soil.

The dry matter content of slurry may vary considerably depending on its origin (McAllister, 1963). These variations may arise through differences in the amount of drinking water available to pigs, the use of wet feed systems or the use of wash water in the piggery. In addition, water may be added to the slurry deliberately to facilitate pumping and spreading. From experience, slurries with a dry matter content of 10% or greater tend to dry out quickly on the surface of the soil and herbage, with little apparent movement into the soil except at very high application rates. It therefore appeared important to examine the effect on copper, zinc and manganese concentration in herbage of applying slurry diluted to varying degrees with water since the liquid fraction of slurry and possibly also the colloidal material might be carried further into the soil towards plant roots.

3.02. Experimental sites, soils and slurry used.

The investigations were carried out on two sites on the E.S.C.A. farms, Midlothian. The sites, measuring 30 x 30 m were located on uniform areas of perennial ryegrass (PRG) swards adjacent to sites A and B used in the investigation previously described (Chapter 2, this thesis). These sites are referred to as A1 and B1, site A1 being located on soil of the Darvel series, Darvel Association and B1 on soil of the Winton series, Rowanhill Association. The characteristics of these soils are described in Appendix I.

Copper-rich pig slurry was obtained from under-floor storage tanks in the fattening house at the E.S.C.A. piggeries, Easter Howgate, Midlothian. The pig rations were supplemented with copper at the rate of 200 mg Cu/kg air dry diet in 1972 and 180 mg/kg in 1973. Approximately 3000 l of slurry were transferred to bitumen-lined, glass-fibre tanks on each site in May 1972 and 1973; the tanks were tightly covered with polythene sheeting.

3.03. Treatments applied.

Twenty blocks measuring 4 x 2 m and spaced 1 m apart were marked out on the established PRG sward on each site. Four treatments were applied on each site, each treatment being replicated five times in a randomised complete block design. The treatments (T) were:

T1 - control, no slurry applied; mineral fertiliser supplying 80 kg nitrogen (N), 18 kg phosphorus (P), and 33 kg potassium (K) ha^{-1} in each of 4 applications in 1972 and 107 kg N, 24 kg P and 44 kg K ha^{-1} in each of 4 applications in 1973.

T2 - 50,000 l copper-rich pig slurry/ha, undiluted; applied 4 times in 1972 and 3 times in 1973.

T3 - 50,000 l copper-rich pig slurry/ha diluted with 50,000 l of water; applied 4 times in 1972 and 3 times in 1973.

T4 - 50,000 l copper-rich pig slurry/ha diluted with 100,000 l of water; applied 4 times in 1972 and 3 times in 1973.

The mineral fertiliser, S.A.I. No. 2 (Scottish Agricultural Industries, Leith, Scotland) was applied at rates to provide 66% of the maximum amount of N which would be required in one year (500 kg N/ha; MAFF, 1976) for production of a grass crop to be harvested rather than grazed; this rate of application supplied adequate P and K for grass production. The pig slurry was applied at a rate to supply N at approximately 4 times the maximum yearly requirement of the grass crop for this nutrient, a situation which might well occur on pig units with inadequate land available for utilisation of the excreta as a fertiliser. The amounts of the major nutrients and the trace elements Cu, Zn, Mn and Fe added to the soil by these treatments are shown in Table 3.1., the values having been calculated from data regarding the concentration of these constituents in the slurry and mineral fertiliser given in Appendix VI.

Herbage on all plots was trimmed back to ground level using a motor-mower prior to each application of slurry or mineral fertiliser, in order to minimise foliar contamination. The

TABLE 3.1.

Major nutrients, trace elements and slurry D.M. applied to established PRG sward in mineral fertiliser or 50,000 l slurry/ha

	SLURRY						MINERAL FERTILISER	
	Site A1			Site B1			Sites A1, B1	
	1972	1973	TOTAL	1972	1973	TOTAL	1972, 1973	TOTAL
N (kg/ha)	1420	705	2125	1540	735	2275	320	640
P "	460	330	790	480	260	840	72	144
K "	580	615	1195	600	615	1215	132	264
D.M. (tonnes/ha)	16.6	21.5	38.1	20.2	23.3	43.5	-	-
Cu (kg/ha)	12.6	11.7	24.3	15.2	13.2	28.4	0.01	0.02
Zn "	10.4	10.6	21.0	12.9	11.3	24.2	0.04	0.08
Mn "	8.8	9.4	18.2	10.4	10.1	20.5	0.03	0.05
Fe "	169.5	170.2	339.7	230.7	190.7	421.4	0.17	0.34

mineral fertiliser was dissolved in water to give a liquid application rate of 50,000 l/ha. The treatments were applied starting in May 1972 and 1973, with a period of approximately 6 weeks between applications in each year; application was by watering-can with a spreader plate attached to the spout. Since 100,000 l/ha is equivalent to a 1 cm layer over a hectare, the treatments were applied to the plots slowly over a period of 2 days to avoid run-off. As slurry was withdrawn from the tanks at each application 4 x 250 ml samples were taken and stored in sealed polythene containers at -15°C for analysis.

Herbage samples were cut approximately 5 cm above ground level using stainless-steel shears when the grass regrowth reached grazing stage at 15 cm in height (stage 1) and again at ear-emergence stage (stage 2). The sample from each plot comprised of 10 sub-samples taken at random within each plot, giving a total sample weight of approximately 200 g of fresh grass. Grass samples obtained at ear-emergence stage, after the final slurry application in the second year of the experiment, 1973 were dried at 80°C to constant weight to determine D.M. content. The grass from an area of 1 x 3 m from the centre of each plot was then harvested within 2 days and weighed to determine yields of fresh material and D.M.

Soil samples were obtained from each plot using a screw auger at depths of 0 to 15 and 15 to 30 cm in December 1972, 3 months after the final slurry application in that year. Since it was found that slurry solids which had accumulated on the soil surface and the top 1 to 2 cm of soil were lost on withdrawing the auger from the soil, samples obtained in 1973 were taken using a 7.5 mm diameter steel tube core sampler. The core samples were taken in December 1973 at depths of 0 to 7.5 and 7.5 to 15 cm, with samples at 15 to 30 cm depth taken by screw auger; 6 cores or 8 auger-sub samples were combined to form the plot sample for analysis. In addition, the surface 2.5 cm of soil plus slurry debris was sampled at 8 points chosen at random within each plot and the sub-samples combined for analysis in 1973. Further core samples were taken at depths of 0 to 7.5 and 7.5 to 15 cm 10 months after the final slurry application of 1973.

3.04. Analytical procedures.

The method for determination of N, P and K in slurry samples is given in Appendix III. The concentrations of Cu, Zn, Mn and Fe in slurry and herbage were determined by the methods described in Appendix IV. Copper in soils was extracted using 0.02 M EDTA (pH 4.2) as the quantity of copper removed by this extractant has been found to be correlated to crop uptake (Hendriksen and Jensen, 1958). Soils were also extracted with 1 N HCl as this extractant has been observed (this thesis, chapter 3) to give complete extraction of the copper in pig slurry. The soil extraction procedures and methods for determination of soil pH and of Cu in the extracts are described in Appendix II.

An analysis of variance was carried out on the results for extractable copper in soils, on herbage Cu, Zn, Mn and Fe concentrations and herbage D.M. content and D.M. yield.

3.05. Results.

(i) EDTA and HCl-extractable copper in the soils.

The mean concentrations of EDTA-Cu in soils sampled at various depths on sites A1 and B1 are shown in Tables 3.2. and 3.3. respectively. In year 1 of the experiment (1972) slurry applications resulted in significantly greater EDTA-Cu levels in soils on all three slurry treatments when compared to the control levels on both soils and at sampling depths of 0 to 15 and 15 to 30 cm. Dilution of slurry with water did not significantly affect the concentration of EDTA-extractable copper in the soils at either sampling depth.

Four months after the final slurry application of 1973, the concentration of EDTA-Cu in the surface 2.5 cm of soil from all slurry treatments on both sites was greater than that of the controls by approximately 50 mg Cu/kg soil. EDTA-Cu concentrations at this sampling depth were not affected by dilution of the slurry. In the complete 0 to 7.5 cm layer of soil sampled in January 1974, the EDTA-Cu concentration in the slurry treatments was greater than that of the controls by 13.5 to 18.7 mg Cu/kg soil and 17.4 to 18.9 mg Cu/kg soil on sites A1 and B1 respectively. At this sampling depth on the light sandy soil of site A1, dilution of the slurry 1:1 or 1:2 with water resulted in greater concentrations of EDTA-Cu in the soil than did undiluted slurry applied in treatment T2.

Slurry application resulted in a small, but significant, increase in EDTA-Cu at a sampling depth of 7.5 to 15 cm on both

TABLE 3.2

Mean concentration of EDTA-extractable copper in soil

Site A1

Date sampled	Sampling depth (cm)	Copper concentration (mg Cu/kg soil) Treatments (T) T1	T2	T3	T4	L.S.D. ($P<0.05$)
19.12.72	0 - 15.0	2.02	2.54	2.72	2.60	0.303
	15.0 - 30.0	1.44	1.80	1.84	1.76	0.181
9.1.74	0 - 2.5	2.44	48.90	58.16	50.24	10.907
	0 - 7.5	2.70	16.20	21.38	20.38	2.173
	7.5 - 15.0	2.04	4.20	4.50	5.04	0.804
	15.0 - 30.0	1.54	2.06	2.40	2.00	0.635
19.7.74	0 - 7.5	2.58	17.36	19.98	21.18	2.005
	7.5 - 15.0	2.12	4.50	4.68	4.88	0.913

TABLE 3.3

Mean concentration of EDTA-extractable copper in soil

Site B1

Date sampled	Sampling depth (cm)	Copper concentration (mg Cu/kg soil)				
		T1	T2	T3	T4	L.S.D. ($P<0.05$)
19.12.72	0 - 15.0	6.32	9.26	10.38	10.94	1.481
	15.0 - 30.0	4.96	5.92	6.54	6.64	1.203
9.1.74	0 - 2.5	7.96	60.24	62.80	59.04	12.422
	0 - 7.5	11.94	29.30	30.68	30.82	4.520
	7.5 - 15.0	10.06	14.08	14.12	16.32	1.783
	15.0 - 30.0	4.88	6.72	6.20	5.64	1.330
19.7.74	0 - 7.5	12.10	30.25	31.04	29.54	4.283
	7.5 - 15.0	10.25	14.28	14.64	16.66	1.801

soils, the increase being greater at the highest slurry dilution rate on site B1 in comparison to the effect of undiluted slurry or slurry plus an equal volume of water. Small increases in soil EDTA-Cu due to slurry treatment were observed at the 15 to 30 cm depth; no effects due to dilution were apparent at this depth.

Soil samples taken in July 1974, 10 months after the final slurry treatment had been applied, showed soil EDTA-Cu concentrations at the same level as those of 6 months earlier.

The percentages of added slurry copper recovered by EDTA extraction of the soils 4 months after the final slurry application are shown, for both sites, in Table 3.4. The percent recovery at each sampling depth was calculated from the difference between the mean EDTA-Cu level in control soils and the mean EDTA-Cu level for slurry treatments T2, T3 and T4, using bulk density data for each soil given in Appendix I.

The extractability or percent recovery of slurry copper from the soils using EDTA was 89 and 88% for soils on sites A1 and B1 respectively. Of the slurry copper added to the soils, 72% was extracted from the surface 2.5 cm of soil plus decaying slurry solids on site A1, while 60% was extracted from this layer on site B1; on the former site, 17% and on the latter site 25% of the slurry copper applied was found to have been leached down the soil profile to depths greater than 7.5 cm.

The levels of copper extracted by 0.02 M EDTA and 1 N HCl from soils which received treatments T1 (mineral fertiliser) and T4 (pig slurry) are shown in Table 3.5.; these soil samples

TABLE 3.4.

The extractability of slurry copper from soils using EDTA

	Sampling depth (cm)	EDTA-extractable Cu (mg Cu/kg soil) Treatment (T) T1	T*	Slurry Cu extracted (kg/ha)**	Extractability of slurry Cu added %
Site A1	0 - 2.5	2.4	52.4	17.5	72
	0 - 7.5	2.7	19.3	17.4	72
	7.5 - 15.0	2.0	4.6	2.7	11
	15.0 - 30.0	1.5	2.2	1.5	6
	0 - 30.0	-	-	21.6	89
Site B1	0 - 2.5	8.0	60.7	17.1	60
	0 - 7.5	11.9	30.3	17.9	63
	7.5 - 15.0	10.1	14.8	4.6	16
	15.0 - 30.0	4.9	6.2	2.5	9
	0 - 30.0	-	-	25.0	88

T* = Mean EDTA-Cu concentration for treatments T2, T3, T4.

** = Calculated as (T*-T1) x kg soil/ha using soil bulk density data, Appendix I.

TABLE 3.5.

Comparison of mean concentrations of slurry copper extracted from soils by 0.02 M EDTA and 1 N HCl

	Extractable Cu (mg Cu/kg soil)				Slurry Cu extracted (mg Cu/kg soil)	
Extractant	EDTA		HCl		EDTA	HCl
Treatment	T1	T4	T1	T4	(T4-T1)	(T4-T1)
Block*						
Site A1						
1	2.9	18.4	4.4	20.3	15.5	15.9
2	2.5	20.5	4.2	21.8	18.0	17.6
3	2.9	23.5	4.5	26.8	20.6	22.3
4	2.5	19.5	4.2	22.0	17.0	17.8
5	2.7	20.0	4.4	22.5	17.3	18.1
Mean					17.68	18.34
± S.E.					1.000	1.061
t					0.453 N.S.	
Site B1						
1	12.5	33.0	14.3	34.5	20.5	20.2
2	11.0	32.2	13.0	35.2	21.2	22.2
3	11.3	29.4	13.1	31.5	18.1	18.4
4	13.2	28.0	14.8	30.4	14.8	15.6
5	11.7	31.5	13.8	34.2	19.8	20.4
Mean					18.88	19.36
± S.E.					1.140	1.116
t					0.301 N.S.	

* Treatment replicate in randomised complete block design.

N.S. Treatment means not significantly different at Students t value < 2.306, ($P<0.05$).

were taken 4 months after the final slurry application of 1973. The amounts of slurry copper extractable with HCl and EDTA did not differ significantly. The pH of soil at a depth of 0 to 15 cm was unaffected by slurry application; the mean values ($\pm$ standard error of difference between means) four months after the final slurry application were 6.3, 6.5, 6.5, 6.3 $\pm$ 0.21 on site A1 and 6.2, 6.3, 6.5 and 6.2 $\pm$ 0.26 on site B1 for treatments T1, T2, T3 and T4 respectively.

(ii) Herbage copper concentrations.

The mean concentrations of copper in perennial ryegrass following application of mineral fertiliser or copper-rich pig slurry to the sward in the first (1972) and second year of the experiment (1973) are shown in Tables 3.6. and 3.7. respectively.

In the first year of the experiment successive applications of copper-rich pig slurry, diluted or undiluted, resulted in significant increases in copper concentration in PRG on each site at growth stages (1) and (2); the maximum concentrations being 15.46 and 24.38 mg Cu/kg D.M., on sites A1 and B1 respectively, during the month of October. Copper concentrations in herbage receiving slurry diluted 1:1 and 1:2 with water were significantly greater than in herbage treated with undiluted slurry at growth stage (1); these treatment differences were evident throughout the period June to November on both sites with the single exception of the early September samples from site B1. Herbage from slurry treatments sampled at ear-emergence stage had a consistently lower copper concentration than the younger herbage sampled at grazing stage. As the season progressed, herbage on the control plots showed a trend towards higher copper contents.

TABLE 3.6

Mean concentration of copper in ryegrass (1972)

(mg Cu/kg DM)

	Treatment (T)	Stage of growth* and sampling date							
		(1) 14 June	(2) 1 July	(1) 24 July	(2) 10 Aug	(1) 9 Sept	(2) 22 Sept	(1) 19 Oct	(2) 24 Nov
Site A1	T1	3.16	4.48	5.44	5.12	5.62	4.78	6.48	6.82
	T2	8.36	5.30	9.04	6.92	8.90	6.38	12.34	12.30
	T3	11.66	5.96	12.06	6.78	14.50	7.84	14.04	13.92
	T4	12.10	6.26	12.16	7.54	15.16	7.32	15.46	13.78
	L.S.D. (P<0.05)	0.769	0.373	1.194	0.445	1.628	0.499	1.307	0.723
Site B1	T1	5.02	5.70	7.26	8.50	8.82	8.36	9.34	8.14
	T2	12.54	6.40	11.26	9.88	19.54	11.56	19.48	11.90
	T3	19.18	6.56	14.74	9.16	20.10	11.78	24.38	12.00
	T4	18.95	6.86	14.10	9.60	18.70	11.40	22.56	11.50
	L.S.D. (P<0.05)	1.859	0.621	1.652	0.680	1.846	1.839	1.682	0.965

* Growth stage (1) = grazing stage; (2) = ear emergence

Slurry applied on 28 May; 7 July; 20 Aug; 29 Sept

TABLE 3.7

Mean concentration of copper in ryegrass (1973/74)

(mg Cu/kg DM)

	Treatment (T)	Stage of Growth * and sampling date							
		(1)	(1)	(2)	(1)	(2)	(1)	(2)	(1)
		9 May	21 June	5 July	25 July	15 Aug	26 Sept	12 Oct	21 May
Site A1	T1	3.56	4.36	4.06	4.36	3.12	4.72	4.80	2.30
	T2	5.24	9.80	7.58	9.04	7.00	8.42	9.46	4.36
	T3	6.96	11.38	7.78	9.28	7.68	8.50	10.80	5.06
	T4	6.76	10.52	7.6 6	9.80	7.72	10.24	12.86	6.02
	LSD (P 0.05)	0.998	0.854	0.447	0.835	0.617	0.529	1.120	0.536
Site B1	T1	8.14	6.44	5.96	9.24	7.22	8.24	7.00	6.08
	T2	10.39	9.58	7.22	11.00	8.40	11.52	10.60	7.08
	T3	10.20	9.58	7.74	11.38	9.16	10.94	11.42	7.48
	T4	10.46	10.22	8.06	11.24	9.42	10.80	11.80	7.10
	LSD (P 0.05)	0.654	0.630	0.719	0.763	0.597	1.813	0.708	0.645

*Growth stage (1) = grazing; (2) = ear emergence
Slurry applied on 4 June; 9 July; 28 Aug.

In the second year of the experiment, herbage sampled at the grazing stage before further slurry had been applied, showed only a small residual effect on copper content from the previous year's slurry treatments.

Application of slurry in the second year of the experiment again increased herbage copper levels above those of the control herbage. The maximum concentrations observed were, however, considerably lower than in the previous year; the concentrations being 12.86 and 11.80 mg Cu/kg D.M. on sites A1 and B1 respectively in October. The increases in herbage copper content with increasing dilution of the slurry applied were also much less marked than in the previous year. Herbage sampled at ear emergence stage was again lower than at the grazing stage after each slurry application.

Although the amount of copper added to the soil increased at each slurry application, copper concentrations in herbage at growth stage (1) were only slightly higher at the end of each season than after the first application of slurry in that season. At 9 months after the final slurry application, copper levels in herbage from slurry treated plots remained significantly greater than the control levels on each site.

Herbage dry matter content, yield of dry matter and uptake of slurry copper are shown in Table 3.8. for the crop of PRG harvested at ear emergence stage, 6 weeks after the final slurry application in 1973. Herbage D.M. content was found to be significantly lower in slurry treatments than in control herbage on site A1 only, the values being 19.2 and 17.6 g D.M./100 g fresh

TABLE 3.8.

Herbage dry matter content, yield of dry matter and uptake of slurry copper by a single crop of perennial ryegrass

Treatments (T)	T1	T2	T3	T4	LSD (P<0.05)
Slurry:water ratio	-	1:0	1:1	1:2	
Site A1					
Herbage D.M. content (g/100 g)	19.2	17.6	17.3	17.6	0.76
D.M. yield (tonnes/ha)	2.24	1.56	2.02	2.06	0.39
Uptake of slurry Cu (g/ha)*	-	7.3	12.1	16.6	-
Site B1					
Herbage D.M. content (g/100 g)	18.3	18.1	17.9	17.4	N.S.
D.M. yield (tonnes/ha)	2.05	1.44	1.68	1.75	0.24
Uptake of slurry Cu (g/ha)*	-	5.2	7.4	8.4	-

LSD = Least significant difference between treatment means.

N.S. = Treatment means not significantly different.

* Uptake calculated as (T-T1) x D.M. yield where T = T2, T3 or T4.

material for treatments T1 and T4 respectively. Yield of D.M. was significantly reduced by undiluted slurry but not by diluted slurry on site A1, whereas all slurry treatments on site B1 reduced D.M. yield in comparison to yield with mineral fertiliser. The reduction in yield was found to be greatest in herbage which had received undiluted slurry. The increase in copper concentration of slurry treated herbage above the control level was calculated to have accounted for the removal of 7.3 to 16.6 g Cu/ha and 5.2 to 8.4 g Cu/ha from the soil on sites A1 and B1 respectively; total addition of slurry copper to the former site was 24.3 kg/ha and 28.4 kg/ha to the latter site.

(iii) Herbage zinc concentrations.

The mean concentrations of zinc in ryegrass following application of mineral fertiliser or pig slurry to the PRG sward in the first and second years of the experiment are shown in Tables 3.9. and 3.10. respectively.

Zinc concentrations in herbage receiving slurry or mineral fertiliser showed a trend towards higher concentrations as the season progressed in each year.

Slurry application significantly increased herbage zinc concentrations above the control levels at grazing stage on each site in both years; at ear emergence stage, however, the effect of slurry was less marked. The maximum concentrations of zinc observed in the first year were 48.8 and 56.4 mg Zn/kg D.M. in November on sites A1 and B1 respectively. Although the total amount of zinc applied to the soil in the second year was

TABLE 3.9

Mean concentration of zinc in ryegrass (1972)

(mg Zn/kg DM)

	Treatment (T)	Stage of growth* and sampling date							
		(1) 14 June	(2) 1 July	(1) 24 July	(2) 10 Aug	(1) 9 Sept	(2) 22 Sept	(1) 19 Oct	(2) 24 Nov
Site A1	T1	22.9	26.6	37.5	36.1	29.1	27.9	37.9	42.1
	T2	40.2	40.4	37.7	35.1	28.0	30.3	42.8	45.9
	T3	42.4	30.6	41.8	36.1	33.7	31.5	42.8	48.6
	T4	45.2	32.4	47.3	39.8	38.8	32.7	46.0	48.8
	L.S.D. ($P<0.05$)	3.17	2.62	3.94	N.S.	1.49	1.88	2.50	4.06
Site B1	T1	24.1	32.4	37.2	40.3	41.0	38.2	49.9	51.7
	T2	36.7	33.3	46.2	37.5	46.2	42.3	55.0	51.1
	T3	42.8	33.1	44.2	38.5	48.4	43.5	55.9	53.2
	T4	41.0	31.0	43.7	37.7	46.5	41.5	56.3	56.4
	L.S.D. ($P<0.05$)	4.05	N.S.	7.27	N.S.	4.31	4.23	4.13	N.S.

* Growth stage (1) = grazing stage; (2) = ear emergence

Slurry applied on 28 May; 7 July; 20 Aug; 29 Sept

TABLE 3.10

Mean concentration of zinc in ryegrass (1973/74)

(mg Zn/kg DM)

	Treatment (T)	Stage of Growth * and sampling date							
		(1)	(1)	(2)	(1)	(2)	(1)	(2)	(1)
		9 May	21 June	5 July	25 July	15 Aug	26 Sept	12 Oct	21 May
Site A1	T1	18.7	26.6	27.0	34.7	22.6	35.1	36.2	18.2
	T2	21.1	42.1	34.9	45.9	31.8	41.1	41.9	26.5
	T3	24.9	44.1	37.3	42.5	35.8	38.2	43.5	30.3
	T4	26.5	43.5	40.6	41.4	38.5	43.2	44.2	33.0
	LSD (P 0.05)	1.47	1.63	2.90	1.76	3.42	1.81	2.46	1.76
Site B1	T1	34.1	28.8	28.4	35.6	25.1	35.2	35.9	27.0
	T2	43.0	40.3	36.5	44.9	33.1	42.1	43.9	34.4
	T3	38.5	40.0	37.5	41.8	39.7	43.0	45.0	34.2
	T4	40.7	39.3	37.0	39.8	35.4	43.7	46.3	35.0
	LSD (P 0.05)	5.23	4.11	5.06	5.52	3.97	6.75	4.62	4.01

*Growth stage (1) = grazing; (2) = ear emergence
Slurry applied on 4 June; 9 July; 28 Aug.

almost double that of the first, the maximum herbage zinc concentrations observed in that year were 44.2 and 46.3 mg/kg D.M. in October on sites A1 and B1 respectively. At 9 months after the final slurry application, zinc levels in herbage from slurry treatments remained significantly greater than the control level.

(iv) Herbage manganese concentrations.

The mean concentrations of manganese in ryegrass following application to the sward of mineral fertiliser or pig slurry in the second year of the experiment are shown in Table 3.11. The mean concentration of manganese in the slurry applied during the season was 482 $\pm$ 18 mg Mn/kg D.M.

Herbage manganese concentrations in control and slurry treatments showed a tendency to increase as the season progressed. In contrast to the situation with copper and zinc, slurry treatments depressed herbage manganese concentrations to levels considerably below those of the controls at the grazing stage on both sites. At ear emergence stage manganese concentrations in herbage from slurry treatments were either lower than or not significantly different from those of the controls. The degree to which the pig slurry was diluted did not produce any consistent effects on manganese concentrations in herbage. The lowest concentrations of manganese observed were 44.4 and 39.7 mg/kg D.M. on sites A1 and B1 respectively. At 9 months after the final slurry application, herbage manganese concentrations in the slurry treatments remained significantly lower than those of the controls.

TABLE 3.11

Mean concentration of Manganese in ryegrass (1973/74)

(mg Mn/kg DM)

	Treatment (T)	Stage of growth * and sampling date							
		(1) 9 May	(1) 21 June	(2) 5 July	(1) 25 July	(2) 15 Aug	(1) 26 Sept	(2) 12 Oct	(1) 21 May
Site A1	T1	73.5	70.9	80.2	116.3	75.3	113.2	152.4	50.0
	T2	44.4	63.5	64.4	79.9	57.8	85.1	103.6	32.3
	T3	52.1	57.9	70.3	66.1	76.4	106.4	129.4	52.7
	T4	64.2	56.0	68.7	63.0	82.8	116.2	129.8	66.9
	LSD (P 0.05)	7.58	3.84	3.68	11.37	9.09	9.85	27.39	5.45
Site B1	T1	70.8	46.9	45.1	69.7	52.4	80.0	88.0	60.2
	T2	57.3	54.2	48.6	68.2	44.5	55.1	57.3	38.7
	T3	52.3	39.7	43.0	55.2	52.6	59.6	57.7	39.2
	T4	58.6	41.4	40.9	56.5	58.2	64.7	72.8	45.0
	LSD (P 0.05)	5.43	4.97	N.S.	6.29	6.08	10.83	9.52	6.30

*Growth stage (1) = grazing stage; (2) = ear-emergence stage.
Slurry applied on 4 June; 9 July; 28 Aug.

TABLE 3.12

Mean concentration of iron in ryegrass 1973/74

(mg Fe/kg DM)

	Treatment (T)	Stage of growth * and sampling date							
		(1) 9 May	(1) 21 June	(2) 5 July	(1) 25 July	(2) 15 Aug	(1) 26 Sept	(2) 12 Oct	(1) 21 May
Site A1	T1	83.7	75.2	77.8	88.9	50.7	97.7	121.2	83.1
	T2	72.2	102.2	79.8	97.3	55.6	96.6	104.2	66.7
	T3	88.4	118.6	85.5	91.2	54.6	89.3	117.4	72.5
	T4	87.0	107.0	84.5	99.0	54.9	80.8	114.6	73.8
	LSD (P 0.05)	8.28	10.50	6.10	8.98	N.S.	13.07	N.S.	7.98
Site B1	T1	135.2	63.8	47.5	86.2	77.4	81.4	126.0	122.6
	T2	170.2	98.5	55.8	92.7	79.3	90.3	113.2	114.6
	T3	120.0	98.3	56.4	83.9	85.3	82.6	128.6	100.0
	T4	125.6	97.9	60.0	84.3	84.0	76.8	124.8	100.1
	LSD (P 0.05)	33.12	6.91	6.75	N.S.	N.S.	8.63	N.S.	N.S.

* Growth stage (1) = grazing stage; (2) = ear-emergence stage
Slurry applied on 4 June; 9 July; 28 Aug.

(v) Herbage iron concentrations.

The mean concentration of iron in the pig slurry applied during the second year of the experiment was 7909 mg Fe/kg D.M. on site A1 and 8171 mg Fe/kg D.M. on site B1.

Although small but significant differences between Fe concentrations in control and slurry treated herbage were observed, (Table 3.12.) slurry treatment did not consistently increase or decrease herbage Fe concentrations.

3.06. Discussion

A dominant feature of the literature regarding the behaviour of copper and other heavy metals in soils is the extent to which these elements are 'fixed' or retained by the various soil components, resulting in low mobility in the soil profile. Jones and Belling (1967) have shown that the native copper in soils has an extremely low mobility, while Purves (1972) has reported that copper and zinc added to soils in sewage sludge are not substantially reduced in concentration by leaching with large volumes of water. As a result of its low mobility, any addition of copper to the soil in excess of crop uptake is likely to result in the accumulation of this element in the soil. The present study provides evidence that the continued disposal of copper-rich pig slurries on agricultural land may lead to enhanced concentrations of this element in the soil.

Repeated applications of copper-rich pig slurry to ryegrass swards, resulting in the addition of 24.3 and 28.4 kg Cu/ha to the soils of sites A1 and B1 respectively, produced a marked increase in EDTA-extractable copper in the soils. Although the distribution of slurry organic matter in the soil profile was not investigated, slurry solids were observed to have accumulated on the soil surface. The concentrations of EDTA-extractable copper, in excess of 50 mg Cu/kg, in the surface 2.5 cm layer of soil plus slurry solids after the final application of slurry are extremely high when compared to the mean level of 11.5 $\pm$ 2.0 reported by Purves (1970) for a number of uncontaminated rural soils.

The concentrations of EDTA-Cu in the 0 to 15 cm layer of soil were apparently only slightly increased above the control levels by slurry at the end of the first year of the experiment, whereas the levels were found to be considerably enhanced after the final slurry application in the second year. Since losses of surface soil and slurry solids were observed to occur on withdrawal of samples using a screw auger, the samples of topsoil obtained by this method in the first year of the experiment were probably not representative of the complete 0 to 7.5 cm layer of soil, resulting in an under-estimation of extractable copper. No calculations were therefore carried out to estimate the percent extractability of slurry copper added to the soils in 1972.

The finding that, in slurry treated soils, the EDTA-Cu levels in the 7.5 to 15 cm layer were greater than in soils receiving mineral fertiliser only would suggest that slurry copper added to the soil surface may move down the profile to some extent. There was, however, little evidence of movement of copper into the 15 to 30 cm layer.

The effect of slurry dilution on EDTA-Cu levels in soil at depths of 0 to 7.5 and 7.5 to 15 cm observed in samples from site A1 cannot readily be explained with the information available. Application of 100,000 l of water/ha is equivalent to a 1 cm layer over this area and may possibly have resulted in larger amounts of slurry copper being carried down to the 7.5 to 15 cm layer in the freely draining, sandy soil of site A1 than would occur with undiluted slurry on this site or with diluted slurry on the imperfectly draining soil of site B1. However, without information on changes in soil organic matter contents arising from slurry application, it is impossible to explain how dilution of slurry increased the EDTA-Cu level in the 0 to 7.5 cm layer of the sandy soil of site A1. It would appear more likely that the EDTA-Cu levels would be reduced by slurry dilution due to the increased likelihood of surface run-off. Since no differences in soil pH were observed between treatments on either site, the dilution effect cannot be explained on the basis of increasing extractability of copper with decreasing pH as reviewed by Hodgson (1963).

The extractability of slurry copper in slurry treated soils was estimated from the difference in EDTA-extractable copper between control and slurry treated soils. Although 1N HCl was found to extract larger quantities of copper from both control and slurry treated soils, the difference in amount extracted between the control and slurry treated soils did not differ significantly from that obtained using EDTA. Ideally, the differences in total soil copper should have been determined. However, facilities for perchloric acid digestion of the soils were not available and it seemed unlikely that copper added to the soil in slurry would enter a soil copper fraction not extractable with the strong acid, HCl.

The accumulation and low mobility of slurry copper in soils has been demonstrated by the finding that 88 and 89% of the added copper was EDTA-extractable from the 0 to 30 cm layer of the soils 4 months after slurry applications were terminated and that the percent extractabilities showed no signs of decreasing when the soils were sampled after a further 6 months. Although the use of a trowel in sampling the surface 2.5 cm layer of soil plus slurry solids is likely to have been unsatisfactory in terms of very accurate control over depth of sampling, the procedure was intended only to give an indication of the copper concentrations at the soil surface. Comparison of the percentage of added slurry copper extracted from this surface layer with the values as determined on core samples would suggest that a large proportion of the added slurry copper had remained in the region of the soil surface. The percent extractability figures have also indicated that a greater quantity of the slurry copper moved downwards to the 7.5 to 15 cm layer in the clay-loam soil of site B1. This is contrary to what might be expected in terms of percolation from the knowledge that the clay-loam soil of site B1 is not as freely draining as that of site A1. However, the possibility exists that anaerobic conditions may have occurred more frequently in the imperfectly draining soil than in the sandy soil thus increasing the mobility of copper through effects on soil pH and redox potential as suggested by Ponamperuma (1972).

In view of the extremely small amounts of copper removed from the soil by the final crop harvested from each site, the total crop uptake of copper over two seasons would account for only a minute fraction of the remaining 10% of slurry copper added to the soil, but apparently not extracted. Since an extractable level of 2.5 mg Cu/kg soil in a 7.5 cm layer over 1 hectare is approximately equivalent to 10% of the slurry copper added per hectare on each site, minor losses of slurry solids or surface soils containing high concentrations of copper might easily account for the remaining 10% of slurry copper not extracted. Such losses might occur during soil sampling or possibly in soil processing as a proportion of dried slurry solids are fibrous in nature and likely to be sieved out of the soil to some extent.

From the results discussed, it would appear that the application /ha of a total of 350,000 litres of slurry from pigs fed rations containing 200 mg Cu/kg diet, may increase the level of EDTA-extractable copper in a 15 cm depth of soil by 19 to 23 mg Cu/kg soil. (EDTA-Cu increases obtained from summation of the differences between EDTA-Cu levels in control and slurry treated soils at the 0 to 7.5 and 7.5 to 15 cm sampling depths in 1973, Table 3.4.). Such a rate of slurry application to soils with EDTA-extractable copper levels of 9 to 11 mg/kg soil would increase the extractable level of this element in soil to 30 mg/kg, which has been reported by Purves (1977) to adversely affect the growth of clovers. The implications of soil contamination with slurry copper will be discussed at length in a later section of this thesis.

The results of this investigation clearly demonstrate that a single application of copper-rich pig slurry to an established PRG sward may markedly increase the concentration of copper in the herbage. Cutting the sward back close to ground level by motor-mower prior to slurry application was found to be effective in preventing surface contamination of the herbage. The increases in herbage copper concentration were therefore due to uptake via the root system.

Dilution of the slurry initially containing 8 to 12% D.M. in 1972 and 13 to 17% D.M. in 1973 resulted in greater concentrations of copper in the herbage at grazing stage than did undiluted slurry. Since Stewart (1968) has observed an increase in crop yield and crop uptake of nitrogen as a result of dilution of pig and cattle slurries, it might be suggested that the dilution of copper-rich pig slurry results in copper in soluble or colloidal fractions in the slurry being carried further into the soil towards the plant root system than would occur with undiluted slurry. The amounts of copper required for the increase in herbage copper content observed with slurry dilution would be extremely small in view of the fact that total uptake of copper in the final crop of herbage harvested in 1973 was only 5 to 16 g/ha (Table 3.8.). McGill, Jackson and Swinburn (1975) have reported concentrations of up to 5 mg soluble copper/l in pig slurry, a concentration which would be more than sufficient at the rates of application used in this investigation, to provide the amounts of copper required for the increased plant concentrations resulting from dilution of the slurry. The suggestion earlier that the mobility and possibly also availability of copper added to the soil may be increased as a result of the development of anaerobic conditions, might also serve to explain the herbage copper increases since Burford (1976) has reported the development of anaerobic conditions in soil following an extremely heavy application of slurry.

The concentration of copper in herbage from slurry treatments was found to be lower at ear-emergence stage than at grazing stage after each application of slurry except the final one in each season. The reasons for this effect may have been two-fold:

(a) Slurry copper added to the soil may rapidly have become less available for crop uptake, especially if the fraction of slurry copper taken up by plants is that in true solution; copper in solution would be expected to be rapidly adsorbed by soil surfaces following microbial decomposition of any soluble complexing agents in the slurry solution.

(b) Increasing maturity in grasses and other Gramineae is accompanied by decreasing concentrations of copper in the plant D.M. (Davey and Mitchell, 1968).

In addition to the differences in copper content observed at growth stages (1) and (2), a seasonal effect on herbage copper content was apparent with copper concentrations in control and slurry treated herbage at a particular stage of growth being greatest in October, at the end of the growing season. The seasonal pattern in herbage copper content was similar to that described by Fleming and Murphy (1968).

It is also interesting to note that repeated applications of slurry over a period of two years did not have an additive effect on herbage copper concentration, the herbage copper content at grazing stage after the seventh slurry application being similar to that after the first application. The reason for this is not readily apparent but might possibly be explained by a rapid decrease in availability of slurry copper as mentioned earlier in relation to the growth stage effects or by the possibility that only a small proportion of the slurry copper is readily available to plants. A further and more likely explanation might involve the control mechanism in grasses which has been reported to limit the transport of copper from roots to tops (Jarvis and Jones, 1979).

Thus, in addition to the total amount of slurry copper applied, the results of this and the previous experiment would indicate that soil characteristics, dry matter content of the slurry as applied, and the plant species present may also be important factors governing the concentrations of copper in herbage in a slurry treated sward. Furthermore, the copper content of slurry treated herbage was shown to vary considerably in relation to stage of growth.

There appeared to be a residual effect from slurry copper in the soil when herbage was sampled 9 months after the final slurry application. The herbage from the slurry treatments on the heavy clay-loam soil (Site B) was found to contain copper, at a maximum, 1 mg Cu/kg D.M. greater in concentration than the control grass. However, slurry treated herbage on the light sandy soil (Site A1) contained 6.02 mg Cu/kg D.M. in comparison to the control level of 2.3 mg/kg D.M. Thus the herbage from slurry treated plots on each site contained similar concentrations of copper, suggesting that the availability of slurry copper in the soils had declined to a level at which the PRG could limit transport to the aerial parts, maintaining the copper concentration in the tops at 6 to 7 mg/kg D.M. With regard to this suggestion, it would have been interesting to have examined the copper content of the roots of grass from slurry and mineral fertiliser treated plots.

The influence of applied slurry on the concentration of copper in herbage, at grazing stage, 9 months after the final application was not as great as that observed in herbage at the same stage of growth 3 to 4 weeks after each individual application. Since soil EDTA-Cu had not shown a decline 9 months after the final application, it would appear that EDTA is not a particularly suitable extractant if extractable levels in soils are to be used to predict concentration of copper in or uptake of copper by grasses growing on slurry treated soils. Owing to the ability of the Gramineae to limit translocation of copper from roots to tops, it may not be possible to correlate copper content of the grasses with amounts of chemically extractable copper in soils.

The application of pig slurry to the PRG swards consistently increased herbage zinc concentrations at grazing stage to levels greater than those of the controls although the increases, in relation to the control level, were not nearly as great as with copper. In contrast to the findings with copper, the effect of dilution of pig slurry on zinc content of the herbage was inconsistent, resulting in greater increases in herbage zinc than obtained with undiluted slurry on only a few occasions. Since many of the reactions of zinc and copper in soils are similar (Hodgson, 1963), these differences in response to dilution of slurry may have arisen through differences in absorption of the metals by the roots or translocation to the tops. Heavy applications of phosphate fertiliser have been found to induce or accentuate zinc deficiency in crops (Olsen, 1972) suggesting that the large amounts of phosphorus added to soils in pig slurry may possibly influence uptake or translocation of the zinc added to soils in slurry. As with copper, stage of maturity and seasonal effects were observed, herbage zinc concentrations showing a small decrease with increasing maturity and an increase towards the end of the growing season. Although zinc was added to the soil at each slurry application, successive crops of grass showed similar concentrations of zinc suggesting that slurry zinc may rapidly become unavailable to crops when added to soil or that plant uptake of the element or translocation to the tops may be under some form of control.

The maximum concentration of zinc observed in the PRG, 56 mg Zn/kg D.M. was observed in October and November 1972, on the heavy clay-loam soil on site B1. Such a zinc concentration is by no means high, being well within the range (35 to 63 mg Zn/kg D.M.) of zinc contents reported for temporary and permanent grass in England and Wales (MAFF, 1974).

Manganese concentrations in the herbage were significantly reduced by slurry application. Singh and Steenberg (1974) have reported that manganese absorption by barley roots may be adversely affected by high concentrations of zinc in the soil. Since pig slurry may contain in excess of 600 mg Zn/kg D.M., the addition of this zinc to the soil may have adversely affected absorption of native manganese in the soil and that added in slurry. Furthermore, it has been shown that organic waste applications to the soil may raise soil pH enough to reduce manganese uptake (Page, 1966). It is interesting to note that Mutlak (1974) has found that manganese was leached from soil to which mixed liquor was added from an oxidation ditch for slurry treatment; no significant amounts of copper were leached from the soils by the liquor. The pH of the liquor was 7.9, similar to that observed by the author for a number of pig slurries (7.8 to 8.2) and may well have solubilised manganese oxide in the soil. Mutlak, however, reported that the mixed liquor did not significantly affect the manganese content of grass grown on soils treated with this material, while liquor from an anaerobic slurry lagoon increased herbage manganese levels when added to soil. Thus it would appear that storage and treatment conditions may influence the effect these waste materials have on soils and the trace element content of crops.

Although, in the present investigation, herbage manganese concentrations were markedly reduced by slurry application, the lowest concentration recorded (44.4 mg Mn/kg D.M.) was greater than the dietary Mn concentration (40 mg/kg D.M.) considered to be adequate for ruminant livestock (A.R.C., 1965).

The effect of slurry application on herbage iron concentrations was found to be inconsistent. Slurry application resulted in either a small increase in herbage iron content or no change at all. The suggestion was made earlier that herbage iron content might possibly be useful as an indicator of surface contamination of herbage with slurry which may contain in excess of 10,000 mg Fe/kg D.M. The range in herbage iron concentrations observed in this investigation would indicate that low levels of contamination could well occur while herbage iron concentrations remain within the normal range (Mitchell, 1957) of 20 to 200 mg/kg D.M. for grasses and clovers.

This investigation has established that addition of copper-rich pig slurry to soils at rates in excess of crop requirements for N, P and K, may result in enhanced copper concentrations in herbage. The herbage copper contents reported in this and other studies (Batey et al, 1972; Lawrence and Gibbs, 1973; Kneale and

Howell, 1974) must be considered a potential toxicity hazard to sheep in view of the numerous reports (Hogan, Money and Blayney, 1968; Suttle, 1968; Adamson, Valks, Appleton and Shaw, 1969; Buck, 1970) indicating that copper toxicity may occur in sheep maintained on diets containing 15 mg Cu/kg D.M. or less.

CHAPTER 4.

STUDIES ON THE PARTITION OF COPPER BETWEEN THE SOLID AND LIQUID PHASES IN PIG SLURRY, THE DESORPTION OF COPPER FROM DRIED SLURRY SOLIDS AND THE FATE OF THE COPPER IN THE SOLUBLE COPPER FRACTION FROM SLURRY WHEN ADDED TO SOILS.

4.01. Introduction.

Within two weeks of applying copper-rich pig slurry to a perennial ryegrass sward, the copper concentration in the herbage was found to have increased to a level markedly greater than that in herbage from a sward receiving mineral fertiliser only (Chapter 3, this thesis). In the same investigation, it was reported that the residual effect of slurry copper on herbage copper content 9 months after the final slurry application was small in comparison to the effect observed in herbage, at the same stage of growth, 2 to 3 weeks after slurry application.

These findings would suggest that there may be a fraction of copper in pig slurry which is readily available to plants in the short term but which may become unavailable or decrease in concentration in the soil in the long term. Such a fraction is most likely to be either in the aqueous phase or in colloidal material which could be carried down through the soil to the root system by the aqueous phase.

The forms of copper in pig slurry and factors affecting their availability to plants have received little study. At the time the investigations reported in this thesis were initiated, the literature contained only one reference to the form in which copper might be present in slurry. From microbiological studies carried out on the faeces of pigs fed rations containing 250 mg

Cu/kg D.M., Andrla (1964) speculated that hydrogen sulphide of bacterial origin might result in the formation of the black and insoluble copper II sulphide in the excreta. Braude (1965) observed that the faeces of copper supplemented pigs were darker than those from unsupplemented pigs and suggested that the black colour of the high-copper excreta would tend to support the theory that the copper is present as the sulphide.

During the present investigations, 1 N hydrochloric acid used for extracting copper from soils was inadvertently added to dried slurry solids. The brown/black colouration of the solids was observed to change to a straw colour. Since CuS is insoluble in dilute HCl (Cotton and Wilkinson, 1966) this observation suggested that the copper in the dried solids was not present as the sulphide.

Copper is taken up by plants via the soil solution (Viets, 1962). From a study of the distribution of copper amongst the various 'pools' in soil, McLaren and Crawford (1973a) suggested that the amounts of this element present at any one time in soil solution plus that which is adsorbed on non-specific exchange sites in the soil are likely to be insufficient to maintain adequate plant growth under intensive systems of cropping. These workers postulated that copper bound by inorganic and organic sites in soil (specific sorption) may be in equilibrium with soil solution and hence replenish this pool as copper is removed from it by growing plants. There is, however, no information regarding the extent to which copper enters the various pools when slurry is added to soil. The observed increases in herbage copper following slurry application to pasture would indicate that a proportion of the slurry copper may enter the soil solution pool.

Since the form or state of copper in slurry would be expected to govern its distribution amongst the various pools in soil and its supply for absorption by plants, investigations were carried out to examine:

(a) the partition of copper between the solid and liquid phases in slurry.

(b) the desorption of copper from dried slurry solids and

(c) the effect of addition to soil of (i) the liquid phase from slurry and (ii) copper sulphate solution, on the levels of copper in the soil solution plus exchangeable pool and on the quantities adsorbed on the specific binding sites of inorganic and organic soil constituents.

4.02. Materials and methods used in the fractionation of copper in pig slurry.

A 2 litre aliquot of pig slurry was withdrawn from the field tanks on site A1 (Chapter 3, this thesis) on 4 occasions spaced 4 weeks apart in 1973. The first aliquot was obtained at the time the tanks were filled for the field studies in that season, the slurry having been produced over the 4 week period prior to transfer to the field tanks; the slurry was produced by pigs fed rations containing 200 mg supplementary copper/kg. Each aliquot was fractionated on the day it was obtained.

The liquid phase was extracted from a 1 litre aliquot of the slurry samples using apparatus for the measurement of soil moisture tension (Soil Moisture Equipment Company, Santa Barbara, California, U.S.A.). A 15 bar ceramic extraction plate with a specified average pore size of 0.19 microns was modified and washed as described in Appendix VII since a number of fitments on the plate were made of brass which may be corroded by ammoniacal solutions such as pig slurry. The liquid was expressed from the slurry using compressed nitrogen, at a pressure of 15 atmospheres, from a cylinder.

Slurry solids were separated as follows into two fractions referred to as the fibrous fraction and the small particulate plus colloidal fraction. A 100 g aliquot of each slurry sample was shaken with 500 ml of distilled water and the suspension filtered through a stainless steel screen with a 500 micron mesh. The fibrous material retained by the screen was washed with 200 ml of distilled water and the washings added to the filtrate. The filtrate (approximately 800 ml) containing suspended slurry solids (small particulate plus colloidal fraction) was centrifuged at 10,000 x g for 1 hour and the supernatant decanted.

Slurry solids were dried to constant weight at 80°C and milled to pass a 1 mm screen. The concentrations of copper in the liquid phase, the two solids fractions and the supernatant from separation of the solids were determined by the method described in Appendix IV. The pH of solutions extracted from slurry was measured using a glass electrode and pH meter.

4.03. Materials and methods used to study the desorption of copper from dried slurry solids.

A 1 litre sample of copper-rich pig slurry was obtained from underfloor storage tanks in the fattening unit at the E.S.C.A. piggery, Easter Howgate, Midlothian; the fattening rations fed contained 200 mg supplementary copper/kg. The slurry was dried to constant weight at 80°C and milled to pass a 1 mm screen. Aliquots (0.2 g) of the dried solids were shaken in polythene containers for 18 hours with dilute hydrochloric acid solutions ranging in pH from 6.9 to 1.0 or with ammonium hydroxide solutions ranging in pH from 7.1 to 8.5; there were 4 replicates at each pH level and the HCl and NH_4OH extracting solutions were prepared using 'Analar' grade chemicals (B.D.H. Chemicals Ltd., Poole, England). The equilibrium suspensions were centrifuged at 1500 x g and the pH of the supernatants reduced to less than 5 by addition of hydrochloric acid. The concentration of copper in the supernatants was determined as for HCl extracts of soil (Appendix II) and the copper content of the dried slurry solids determined as described in Appendix IV. The pH of the supernatants was determined using a glass electrode and pH meter.

The extractability of dried solids from a number of samples of copper-rich slurries from the field investigations reported in Chapters 2 and 3 of this thesis was determined using 0.05 M calcium chloride solution and 0.5 M acetic acid (pH 3.6), the reagents used by McLaren and Crawford (1973a) for assessing the quantities of 'solution plus exchangeable copper' and of copper bound to inorganic sites, respectively, in soils. Copper was also extracted from the dried solids using 0.02 M EDTA (pH 4.2), which has been shown to remove this element from soil organic matter (Henriksen and Jensen, 1958). In addition, copper was extracted using the strongly acid reagent, 1 N. hydrochloric acid. For comparison, dried solids from slurry produced by breeding sows fed a ration containing 53 mg Cu/kg was extracted using the above reagents.

An aliquot (0.2 g) of dried solids was shaken for 24 hours with 100 ml of each reagent, each extraction being replicated 5 times. The suspensions were centrifuged at 3,000 x g for 20 minutes. The concentrations of copper in the EDTA and HCl supernatants were determined as described for soils extracts (Appendix II) while copper in calcium chloride (Cu-CA) and acetic acid (Cu-AA) extracts was determined using the procedure given in Appendix V. The pH of the extracts was measured using a glass electrode and pH meter.

4.04. Materials and methods used to study the fate of the soluble copper fraction from slurry when added to soils.

Copper-rich pig slurry was obtained from underfloor storage tanks in the fattening house at the E.S.C.A. pig unit, Easter Howgate, Midlothian; the rations fed contained 200 mg supplementary copper/kg. The liquid phase was extracted from a 1 litre aliquot of the slurry using the method given in section 4.02; the solution contained 3.5 mg Cu/l. The solid residue after liquid removal was dried to constant weight at 80°C and milled to pass a 1 mm screen; the solids contained 651 mg Cu/kg D.M.

The soil used in this study was a topsoil taken at a depth of 0 to 15 cm from land under a perennial ryegrass sward on the E.S.C.A. farms, Bush Estates, Midlothian; it was a freely draining sandy soil of the Darvel series, Darvel Association (The Soils of the Bush Estates, Midlothian, 1969). The characteristics of this soil are given in Appendix I. Soil passing a screen with a mesh size of 500 microns was used in these investigations.

Aliquots of soil (100 g) were weighed out and placed in 200 ml polystyrene containers which had previously been washed with 6 N hydrochloric acid and rinsed with distilled water. The treatments applied to the soils were:

(i) 10 ml distilled water added per 100 g soil

(ii) 10 ml of copper sulphate solution ($CuSO_4.5H_2O$ in water; 3.5 mg Cu/l) added per 100 g soil

(iii) 10 ml of solution (3.5 mg Cu/l) extracted from slurry added per 100 g soil.

There were initially 36 replicates per treatment and 4 replicates were taken from each treatment at 3 or 4 day intervals in order to assess the effect of treatment on the quantities of 'solution plus exchangeable' copper and of copper specifically adsorbed on inorganic and organic sites in the soil. The treated soils were brought to 50% saturation with distilled water and the moisture content maintained at this level for 27 days at room temperature in the laboratory.

The quantities of solution plus exchangeable copper in the soils were assessed by extraction with 0.05 M $CaCl_2$ solution and that bound to inorganic sites was extracted using 0.5 M acetic acid as described by McLaren and Crawford (1973a). Copper bound by organic sites was extracted using 0.02 M EDTA, pH 4.2, as described by Henriksen and Jensen (1958). An analysis of variance was carried out on the levels of copper extractable from the soil by each reagent.

4.05. Results.

The concentrations of copper in the various slurry fractions are shown in Table 4.1. The concentrations of copper in the solutions extracted from the slurry after storage for 4 to 16 weeks showed little variation, the mean value ($\pm$ standard deviation, S.D.) being 4.1 $\pm$ 0.2 mg Cu/l. The pH values for the solutions from slurry stored for 4, 8, 12 and 16 weeks were 7.8, 7.8, 7.7 and 7.6 respectively.

The supernatant liquid obtained from centrifugation of the suspended solids contained 9.4 mg Cu/l and was slightly turbid indicating incomplete sedimentation of the slurry solids.

Two aliquots (100 ml) of solution extracted from slurry were found to contain an aggregated black precipitate after storage for a period of 8 months in stoppered flasks at room temperature; the solutions were those extracted from the slurry stored 4 and 16 weeks and contained 4.2 and 4.1 mg Cu/l respectively. Analysis of the supernatant in these flasks revealed that the concentrations of dissolved copper had decreased

TABLE 4.1.

Concentration of copper in the liquid and solid phases of slurry during storage

Storage time (weeks)	Dry Matter (g D.M./l slurry)			Copper			
				mg Cu/kg D.M.			mg Cu/l
	Whole Slurry	Fibrous fraction	Particulate/ Colloidal fraction	Whole Slurry	Fibrous fraction	Particulate/ Colloidal fraction	Liquid extracted
4	148	121	25	542	105	2415	4.2
8	158	128	28	554	130	2258	3.8
12	155	122	30	549	115	2306	4.2
16	159	127	29	569	138	2317	4.1
MEAN	155	125	28	554	122	2324	4.1
± S.D.	± 4	± 3	± 2	± 10	± 13	± 57	±0.2

S.D. = standard deviation from the mean.

to less than 0.05 mg Cu/l with an associated decrease in pH from 7.8 and 7.6 to 7.2. The solution and precipitates were decanted from the flasks, and each flask washed three times with 25 ml aliquots of distilled water. The possibility that copper from the solution had become adsorbed on the walls of the flask was ruled out after washing with 25 ml of 1 N HCl was found to have removed negligible quantities of copper from the glass.

Addition of 25 ml of 1 N HCl to the black precipitates resulted in immediate dissolution with evolution of hydrogen sulphide gas (H_2S) as shown by test with moist lead acetate paper, the paper turning black due to formation of lead sulphide. On analysis these HCl solutions were found to contain 0.41 and 0.39 mg copper. Thus 98% and 95% of the copper present in the solutions extracted from slurry stored 4 and 16 weeks respectively was recovered from the precipitates, which were also found to contain 2.1 mg manganese and 11.9 mg iron.

The extractability of copper in dried solids from slurries with copper contents ranging from 96 to 770 mg Cu/kg D.M. is shown in Table 4.2. Virtually all the copper was extractable with HCl while EDTA removed on average, 75% of the total amount of this element in the slurry solids. Acetic acid, a weak acid, extracted only 15% and calcium chloride displaced 1 to 4.6% of the slurry copper. There was no apparent relationship between extractability and copper content or period of storage of the slurry with any of the extractants used.

On addition of HCl or NH_4OH solution, the slurry solids became lighter in colour. The solids appeared to act as a buffer,

TABLE 4.2.

The extractability of copper in dried pig slurry solids using 1 N HCl, 0.02 M EDTA 0.5 N acetic acid (A.A.) and 0.05 M calcium chloride solutions as extractants

Total copper content of slurry (mg Cu/kg D.M.)	Storage time (weeks)	Extractability (%)			
		HCl	EDTA	A.A.	$CaCl_2$
96	< 4	102	73	16	1
107	< 4	98	72	19	1
542	8	101	77	15	4
556	12	96	75	15	3
565	16	98	78	13	4
690	< 4	96	72	16	3
730	< 4	102	79	19	5
732	8	98	77	12	3
764	12	100	73	11	2
770	16	99	74	14	4
	MEAN	99.0	75.0	15.0	3.0
	± Standard deviation	2.2	2.6	2.7	1.3

extracting solutions with an initial pH value within the range 3.95 to 8.50 showing an equilibrium pH value of 7.30 after shaking. The relationship between initial and equilibrium pH values of the extracting solutions are shown in Figure 4.1.

The relationship between initial pH of the extracting solution and desorption of copper from the slurry solids is shown in Figure 4.2. Less than 15% of the slurry copper was desorbed between pH 2.9 and 9.1. Desorption of copper increased slowly from pH 9.7 to pH 11 and very rapidly from pH 2.3 to 1.3, desorption being virtually complete (96.5%) at pH 1.35.

The changes in Cu-CA, Cu-AA and Cu-EDTA levels in soils treated with copper sulphate solution or solution extracted from pig slurry are shown in Figure 4.3. Copper sulphate and the solution from slurry resulted in significant increases in the Cu-CA fraction in soil up to 9 days after the treatments were applied; enhanced Cu-CA levels were maintained by the slurry extract, but not by copper sulphate from day 11 to day 27. Cu-AA levels in soils treated with copper sulphate and slurry extract were significantly greater than those in untreated soil throughout the experiment; the effects of slurry extract and copper sulphate on soil Cu-AA levels did not differ significantly. Both copper sources resulted in small increases in soil EDTA-Cu levels.

FIGURE 4.1

Change in pH of hydrochloric acid and ammonium hydroxide extractants after shaking with dried slurry solids.

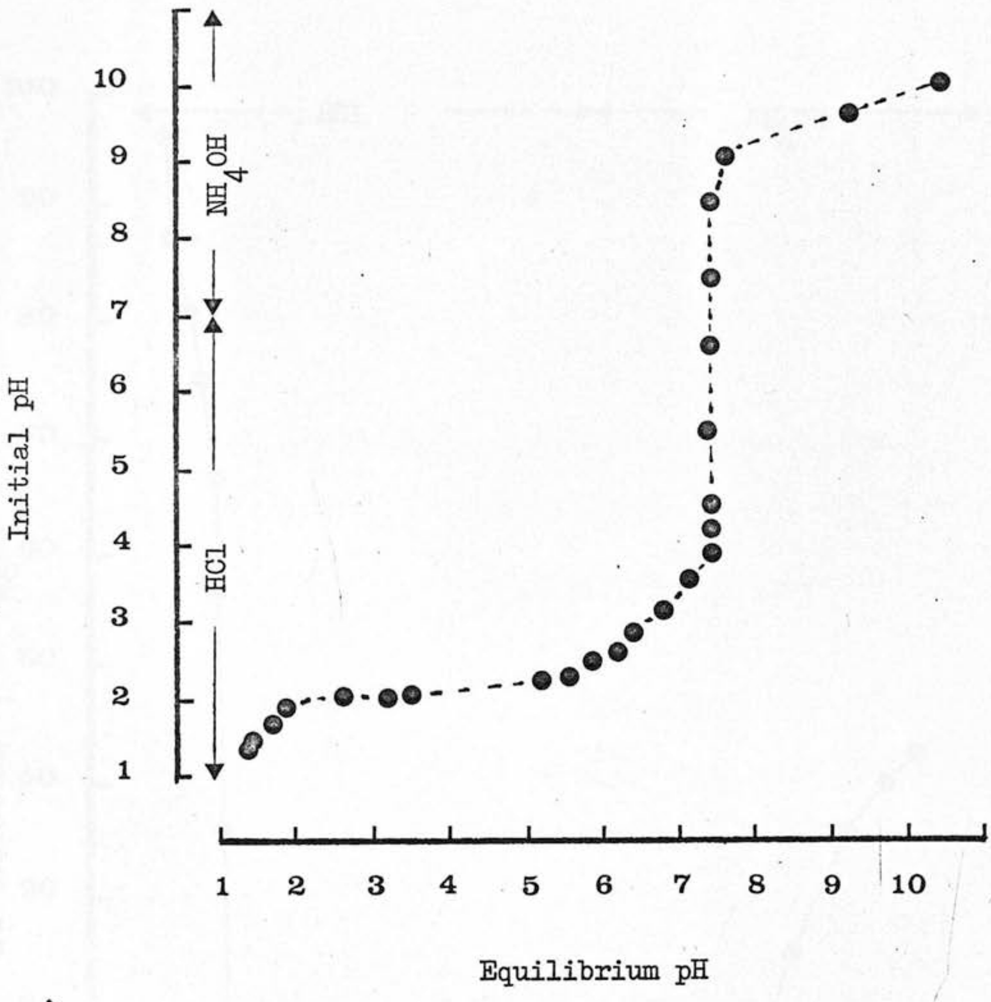


FIGURE 4.2.

Relationship between initial pH of hydrochloric acid and ammonium hydroxide extracting solutions and the desorption of copper from dried slurry solids containing 651 mg Cu/kg D.M.

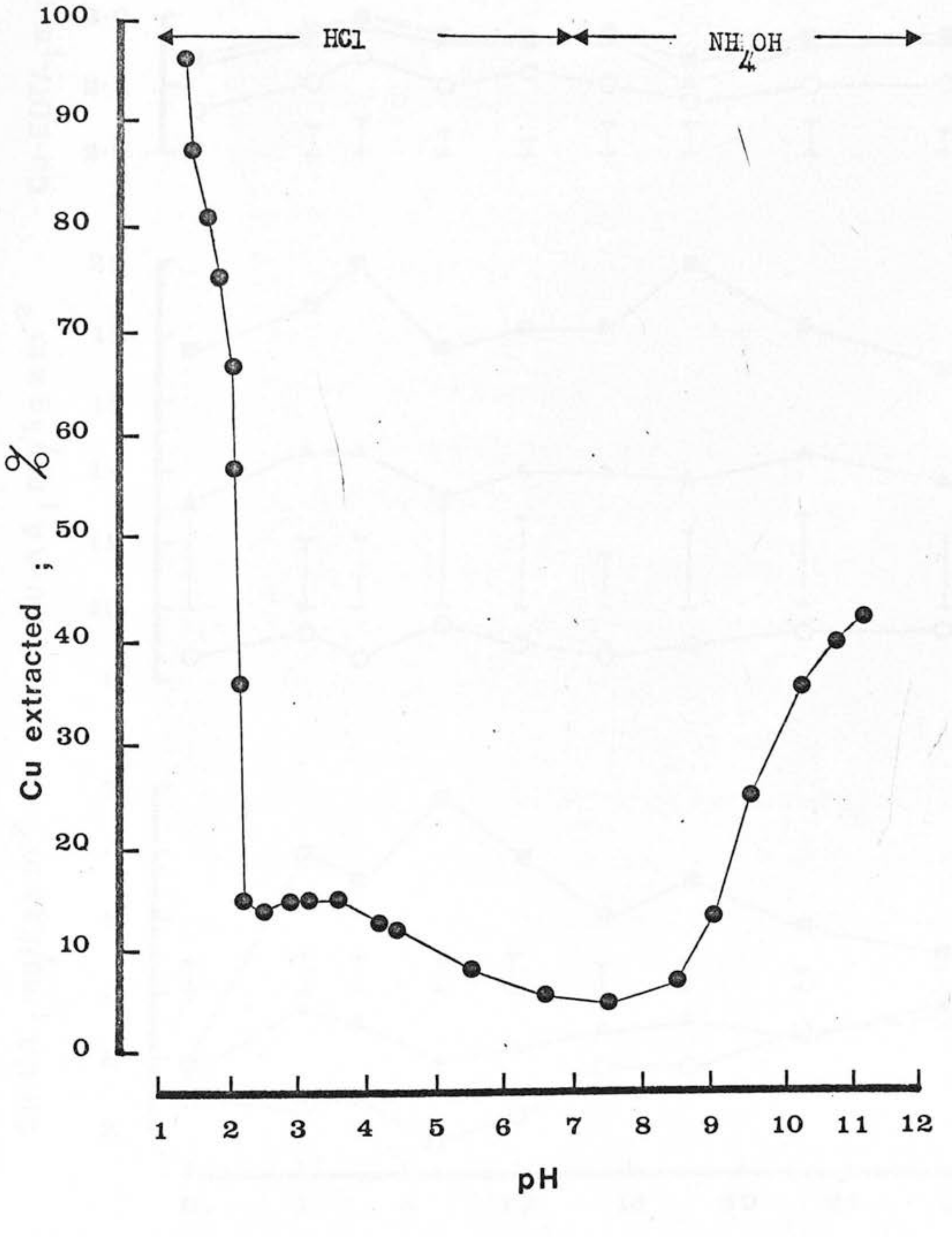
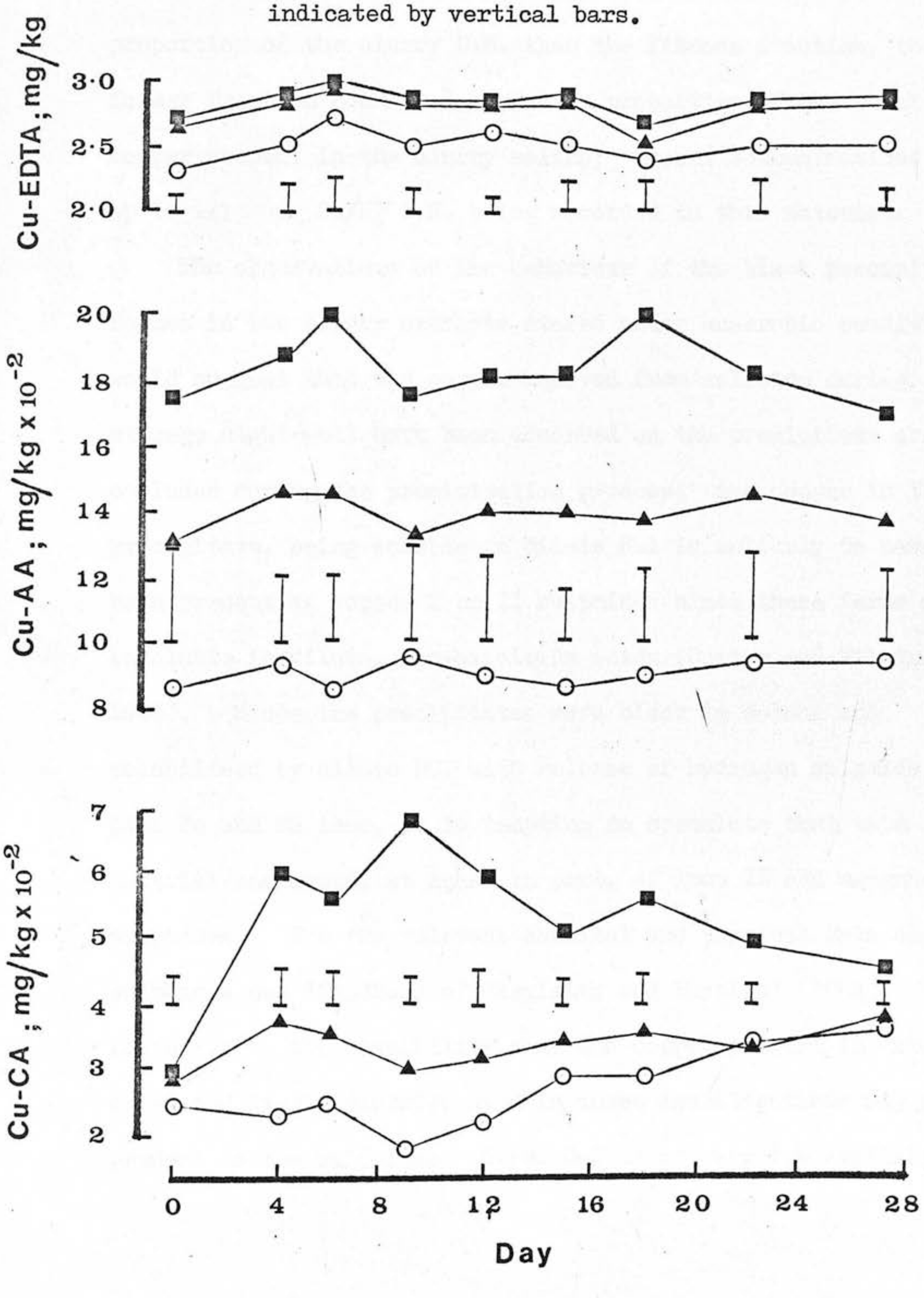


FIGURE 4.3.

Changes in the mean concentration of extractable copper in soil following addition of water (o—o), copper sulphate solution (▲—▲, 3.5 mg Cu/l) or a solution extracted from slurry (■—■, 3.5 mg Cu/l). The least significant difference ($P<0.05$) between mean extractable levels with the reagents 0.05 M calcium chloride (Cu-CA), 0.5 M acetic acid (Cu-AA) and 0.02 M EDTA (Cu-EDTA) are indicated by vertical bars.



4.06. Discussion.

The findings of the present fractionation studies are in close agreement with those of McGill et al (1975) indicating that concentrations of up to 5 mg Cu/l may be present in the aqueous phase of copper-rich slurries. Although the small particulate plus colloidal fractions constituted a lower proportion of the slurry D.M. than the fibrous fraction, the former fraction contained a greater proportion of the total copper present in the slurry solids; copper concentrations of up to 2415 mg Cu/kg D.M. being recorded in this material.

The observations on the behaviour of the black precipitate formed in the slurry extracts stored under anaerobic conditions would suggest that the copper removed from solution during storage might well have been adsorbed on the precipitate or occluded during the precipitation process; the copper in this precipitate, being soluble in dilute HCl is unlikely to have been present as copper I or II sulphides since these forms are insoluble in dilute, non-oxidising acids (Cotton and Wilkinson, 1966). Since the precipitates were black in colour and solubilised by dilute HCl with release of hydrogen sulphide gas plus Fe and Mn ions, it is tempting to speculate that this material consisted, at least in part, of iron II and manganese II sulphides. For the relevant chemical and physical data on these sulphides see 'Handbook of Chemistry and Physics' (1964). There is therefore the possibility that the copper present in the oven dried and liquid slurries used in these investigations may not be present as the sulphide. Since CuS is of very low availability to plants (Hodgson, 1963), the rapid increases in herbage copper observed in the present field studies, those by Batey et al (1972) and Mutlak (1974) would tend to support this suggestion. The quantities of slurry present in the liquid phase would, however, be more than sufficient, at the rates of slurry application used in these studies, to account for the increase in copper uptake observed in herbage following slurry application.

The present investigation has, in addition, shown that copper in slurry solution may increase soil solution plus exchangeable copper to a greater extent and for a longer period of time than copper sulphate. This effect may well be due to the copper being complexed by organic compounds in the extracts since Miller and Ohlrogge (1958) have shown that addition of organic materials to soils may increase the chemical extractability of trace elements through formation of soluble complexes. Although these workers found that water extracts of manure solubilised zinc in soils, plant uptake of this element was decreased. Further research is therefore required to examine the influence of the liquid phase from slurry on plant uptake of copper, especially since the slurry extract increased the soil Cu-AA level which is one of the copper pools in soil considered to be important in maintaining the concentration of copper in soil solution.

The desorption study indicated that copper in dried slurry solids is very strongly bound, little being released until the pH of the medium is reduced to below 2.3 or increased to above 9.7. From this work it is not clear whether the apparent buffering effect of the solids was due to chemical reaction or to formation of charged groups on organic molecules.

Only 3% of the copper in the solids was exchangeable with the Ca^{2+} ion and the low extractability, 15%, with acetic acid would indicate that adsorption of copper on inorganic sites was of minor importance. Since copper was released in significant amounts from the slurry solids at a pH lower than 2.3 in HCl and since 75% was removed by EDTA extraction at pH 4.2, it would appear that copper in the dried solids is bound mainly to organic sites. Further research would however be required to ascertain whether or not copper in liquid slurry is also bound mainly to organic sites.

In contrast Robel and Ross (1975) have reported that virtually all the copper in dried slurry solids is present as the sulphide. These conflicting conclusions would suggest that further research should include an investigation of the influence of storage conditions on the forms of copper in copper-rich pig slurry. The findings of the present study that pig slurry contains a soluble copper fraction and a fraction bound mainly to organic matter may help explain the responses in copper content of herbage observed following slurry application in the field. Furthermore, organically bound copper in slurry is likely to be available to grazing livestock, raising the question as to whether or not sheep grazing slurried pasture or fed diets containing slurry would retain sufficient copper from slurry to give rise to clinical copper poisoning.

CHAPTER 5.

INVESTIGATION OF THE AVAILABILITY TO RYEGRASS OF COPPER ADDED TO SOIL IN SLURRY, IN THE AQUEOUS PHASE FROM SLURRY OR AS COPPER SULPHATE.

5.01. Introduction.

The evidence currently available supports the concept that ion uptake by plant roots is related to ionic activities at the root surface (Baker, 1971, 1973). For most crops the activity or effective concentration of copper at the root surface should be 0.02 to 0.04 mg/l while levels of 0.1 to 0.3 mg Cu/l have been found to be toxic to crop plants (Hewitt, 1952; Smith and Specht, 1953; Struckmeyer et al, 1969). According to the concept of micronutrient pools in soils (Viets, 1962), the fractions of soil copper important in relation to availability to plants are those in equilibrium with the water soluble form. Thus, the solution theory holds that any ion in soil solution is available to the plant if the root is capable of absorbing it and that ions not in solution must pass into solution and move to the root by diffusion or mass flow in water before they may be absorbed.

Copper has been found in the aqueous phase of slurry in concentrations of up to 5 mg/l (Chapter 4, this thesis), which is in close agreement with the values reported by McGill, Jackson and Swinburn (1975). In view of the finding in the present investigations that a major proportion of the copper in pig slurry is very firmly bound to the solids and released in quantity only at pH values less than 4, which is extremely low for agricultural soils, it might be suggested that the fraction of slurry copper responsible for the marked increases in plant uptake may be that which is in solution. In studies reported earlier in this thesis (Chapter 3), the application of 50,000 l/ha was calculated to have increased herbage copper uptake by a maximum of 16.6 g Cu/ha. At this rate of application, the concentration of copper in 'slurry solution' required to provide 16.6 g Cu/ha would be 0.33 mg Cu/l, which is considerably less than that observed in slurries.

Since it has also been shown that copper in the aqueous phase of slurry may produce a short-term increase in soil solution plus exchangeable copper when added to soils under laboratory conditions (Chapter 3, this thesis), it was decided to investigate the effect of addition of this fraction of copper to soil on the copper content of ryegrass grown on the soil. The uptake of copper by ryegrass growing on soil treated with 'slurry solution', slurry and a copper sulphate solution were compared in a pot experiment under greenhouse conditions.

5.02. Soil.

The soil used in this investigation was a topsoil taken at a depth of 0 to 15 cm from land under a perennial ryegrass sward on the E.S.C.A. farms, Bush Estates, Midlothian; it was an imperfectly draining clay-loam of the Winton series, Rowanhill Association (The soils of the Bush Estates, Midlothian, 1969) and contained 0.16 mg acetic acid-extractable copper (Cu-AA)/kg and 6.4 mg EDTA-Cu/kg. The characteristics of this soil are given in Appendix I.

Soil which passed a 4 mm screen was mixed 1:1 with water-washed quartzite gravel sieved to give a particle size range of 2 to 4 mm and 6 kg of the mix placed in 40 polypropylene pots.

Perennial ryegrass seed (Aberystwyth S.23, 0.5 g/pot) was sown on all pots and watered daily thereafter with 150 ml of glass-distilled water per pot.

5.03. Slurry and extraction of slurry solution.

Copper-rich pig slurry was obtained from under-floor storage tanks in the fattening unit at the E.S.C.A. piggeries, Easter Howgate, Midlothian; the fattening rations contained 200 mg supplementary copper/kg. The slurry had accumulated in the tanks over a period of 27 days.

Approximately 15 litres of slurry, withdrawn from the tanks using a polythene bucket, was well mixed and stored at 4°C in 1 litre, screw capped polythene containers. The dry matter content of the slurry, which had a density of 1.045 kg/l, was 24.8 g D.M./100 g and contained 724 mg Cu/kg D.M. The concentrations of the major nutrients N, P and K in the slurry were 0.74, 0.36 and 0.39 g/100 g respectively.

A total of 4 litres of slurry solution were extracted within 4 days of removal of the slurry from the under-floor tanks; the method of extraction was as described in the previous chapter and the extract was stored at 4°C until required. By this method, 72 ml of solution was extracted/100 ml of slurry and the solution contained 0.37 g N, 0.10 g P, 0.28 g K/100 ml and 4.2 mg Cu/l.

The slurry and slurry extract were diluted with distilled water 1:1 (v/v) to ensure even application to the soil.

5.04. Treatments.

The experimental design was a 4 x 2 factorial with 5 replicates. The sources of copper (T) added to the soil at rates (R) were:

T1 no copper added: N, P, K solution; 100 ml/pot (R1) or 300 ml/pot (R2)

T2 diluted slurry; 138 ml/pot (R1) or 414 ml/pot (R2)

T3 diluted slurry extract; 100 ml/pot (R1) or 300 ml/pot (R2)

T4 copper sulphate solution; 100 ml/pot (R1) or 300 ml/pot (R2) plus N, P, K solution; 100 ml/pot (R1) or 300 ml/pot (R2).

The lower slurry application rate (R1) was chosen to add the equivalent of 45,000 l slurry/ha to soil in the pots, a rate similar to that shown (Chapter 3, this thesis) to markedly increase herbage copper content under field conditions. The higher rate (R2) represented an excessive application in terms of nutrient requirements of a grass crop, adding nitrogen at a level (954 kg/ha) twice the maximum yearly requirement

(MAFF, 1976) for ley land. Slurry extract was applied at rates to provide the volumes of solution extractable from the quantities of slurry applied in treatment T2. The copper sulphate solution was identical in copper concentration to the liquid slurry. The solution supplying the major nutrients in treatments T1 and T4 was prepared using Analar grade (B.D.H. Chemicals Ltd., Poole, England) NH_4NO_3, KH_2PO_4 and K_2SO_4 to give N, P and K concentrations identical to those of the slurry extract; the concentration of copper in this solution was less than 0.1 mg/l.

The ryegrass was allowed to grow to 20 cm in height and then removed by cutting at 2 cm above soil level. The treatments were then applied to the surface of the soil, additions taking place over a period of 3 days. The regrowth was harvested twice when it reached a height of 15 cm, the herbage being cut 3 cm above soil level.

5.05. Analytical Methods.

The concentrations of N, P and K in the slurry and slurry extract were determined by the methods given in Appendix III. Copper concentrations in these materials and in herbage dry matter were determined as described in Appendix IV. All soil from each pot was air dried and that which passed a 2 mm screen was well mixed; a 200 g sample was retained for analysis.

Copper was extracted from soil using a 0.5 M aqueous solution of acetic acid, which is thought to remove copper in soil solution and that on mainly inorganic exchange sites (Mitchell, Reith and Johnston, 1957a,b). The extraction procedure is given in Appendix V.

Soil pH was determined in a slurry of soil in distilled water (1:1 w/v) using a glass electrode and pH meter.

An analysis of variance was carried out on the results obtained.

5.06. Results.

The mean concentrations of copper in herbage are shown in Table 5.1. In the first crop copper sulphate solution and slurry increased the copper concentration in the herbage above that of the controls at both levels of addition to the soils. Herbage copper concentration was significantly increased by slurry solution at the higher level of addition only; there was also a significant interaction between copper source and rate of application. By the second harvest, herbage copper concentration at the lower rate of application was increased above the control level by slurry but not by copper sulphate or slurry solution; at the higher level of addition, copper sulphate solution and slurry resulted in elevated herbage copper contents.

The effects of copper source and rate of application on yield of herbage dry matter and uptake of copper are shown in Table 5.2.

TABLE 5.1.

Effect of copper source (T) and rate of application (R) on copper content of perennial ryegrass

Harvest	Copper source	(Cu mg/kg D.M.) Application rate R1	R2
1	T1 Mineral fertiliser	14.3	15.2
	T2 Slurry	15.9	19.2
	T3 Slurry extract	14.2	17.8
	T4 $CuSO_4.5H_2O$	15.4	18.7
	L.S.D. ($P<0.05$)	T, 0.78; R, 0.52;	RxT, 1.04
2	T1 Mineral fertiliser	13.7	13.8
	T2 Slurry	14.3	15.1
	T3 Slurry extract	14.0	14.2
	T4 $CuSO_4.5H_2O$	14.1	14.5
	L.S.D. ($P<0.05$)	T, 0.54; R, 0.38;	RxT, N.S.

N.S. = Means not significantly different.

Slurry, at the higher rate of application, significantly reduced yield of dry matter in the first and second crops. Dry matter yield increased with increasing rate of application in all treatments except with slurry in T2; there was a significant interaction of copper source and application rate on D.M. yield.

The results in terms of copper uptake/pot closely followed those for concentration except in the case of herbage grown on the slurry treated soil where uptake was significantly lower than that for the other treatments at each harvest and each application rate; there was a significant interaction effect of copper source and application rate at the first harvest only.

TABLE 5.2.

Effect of copper source (T) and rate of application (R) on yield of dry matter and copper uptake by ryegrass

Harvest	Copper source	Copper applied mg Cu/pot		D.M. yield (g/pot)		Cu uptake (mg Cu/pot)	
		R1	R2	R1	R2	R1	R2
	mineral fertiliser	<0.02	<0.02	9.1	11.2	0.13	0.17
	slurry	12.96	38.88	8.8	8.3	0.14	0.16
	slurry extract	0.63	1.89	9.2	10.7	0.13	0.19
1	$CuSO_4 \cdot 5H_2O$	12.96	38.88	9.1	10.7	0.14	0.20
	L.S.D. ($P<0.05$)			T, 0.51; R, 0.72; TxR, 1.02		T, 0.006; R, 0.009; TxR, 0.012	
	mineral fertiliser	-	-	8.8	10.9	0.12	0.15
	slurry	-	-	8.4	8.6	0.12	0.13
	slurry extract	-	-	9.3	10.6	0.13	0.15
2	$CuSO_4 \cdot 5H_2O$	-	-	9.2	11.0	0.13	0.16
	L.S.D. ($P<0.05$)			T, 0.59; R, 0.89; TxR, 1.19		T, 0.004; R, 0.006; TxR; N.S.	

N.S. = No significant difference between means (5 replicates)

L.S.D. = Least significant difference

The mean concentrations of acetic acid-extractable copper and soil pH values are shown in Table 5.3. Soil pH was not significantly affected by any of the treatments. Slurry markedly increased soil Cu-AA at both rates of application, while copper sulphate solution increased soil copper, extractable with this reagent, at the higher rate of application only. Soil Cu-AA was unaffected by application of the solution extracted from slurry.

The higher rate of slurry application resulted in a greater concentration of Cu-AA in soil than did the lower rate, the extractable levels being 0.314 and 0.233 mg/kg soil respectively.

5.07. Discussion

The application of slurry to pasture has been shown to increase the concentration of copper in herbage at grazing stage in the absence of foliar contamination, the enhanced copper concentrations declining again as the herbage reached ear-emergence stage (Chapter 3, this thesis). In addition it was shown that slurry diluted with water resulted in greater increases in the concentration of copper in herbage than did undiluted slurry. This effect of dilution, which has also been reported by Stewart (1968) in relation to nitrogen uptake by grass following slurry application, led to speculation that the water applied may possibly carry the soluble copper fraction in slurry further into the topsoil, the solid residues containing the greater proportion of slurry copper remaining on the soil surface.

TABLE 5.3.

Effect of copper source (T) and rate of application (R) on soil pH and mean concentration of acetic acid extractable copper (Cu-AA) in soil

Copper source	Copper applied (mg Cu/kg soil)		Soil pH			Cu-AA (mg Cu/kg soil)		
	R1	R2	R1	R2		R1	R2	
T1 no Cu added	<0.01	<0.01	6.0	6.1		0.160	0.159	
T2 slurry	4.32	12.96	6.2	6.2		0.233	0.312	
T3 slurry extract	0.21	0.63	6.1	6.0		0.165	0.169	
T4 $CuSO_4 \cdot 5H_2O$	4.32	12.96	6.1	6.1		0.166	0.171	
			T	R	TxR	T	R	TxR
L.S.D. (P<0.05)			N.S.	N.S.	N.S.	0.015	0.021	0.031

N.S. = Means not significantly different

L.S.D. = Least significant difference between means

Under the conditions of the present investigation the slurry extract did not affect the concentration or total uptake of copper in herbage at the lower rate of application, which was equivalent to applying 45,000 litres of slurry/ha; this rate of slurry application markedly increased herbage copper concentrations in the earlier field investigations. It would therefore appear that the small amount of copper added in the slurry extract (0.63 mg/pot) was effectively immobilised by the soil since this amount of copper was well in excess of that taken up by grass (0.13 mg/pot) grown with inorganic fertiliser supplying only N, P and K. Further evidence for soil fixation of slurry extract copper was provided by the finding that this lower application rate did not increase the level of acetic acid extractable copper in the soil.

In contrast, slurry extract applied at the higher rate, R2, significantly increased herbage copper content and copper uptake in relation to the control level. Although the application of whole slurry resulted in a significantly greater increase in concentration of copper in herbage than did the equivalent volume of slurry extract, copper uptake from the whole slurry was lower than that from the extract and was associated with a decrease in dry matter yield.

The depression in yield caused by the higher slurry application rate may have been due to a combination of the physical effects of slurry in restricting soil aeration and an increased demand for oxygen in the soil as a result of the large amounts of readily degradable organic matter in the slurry. Burford (1976) has attributed the poor growth of a grass sward to such effects following the application of 550 tonnes of slurry/ha and reported the development of anaerobic conditions with low redox potentials in the soil. Although the availability of copper in soils and uptake by plants may be increased under these conditions (Hodgson, 1963), soil pH was not significantly affected by the treatments applied and slurry did not result in significantly greater herbage copper concentrations than $CuSO_4$ at the first harvest. Since slurry at both rates of application and slurry extract at the higher rate, R2, increased the level of acetic acid extractable copper in the soil, the effect of these copper sources on herbage copper concentration would appear to have been due mainly to the addition to soil of copper available for uptake by the herbage, with no indication of an effect of anaerobic conditions in the soil on availability of the added slurry copper.

The quantities of the major nutrients N, P and K added to the soil in slurry were greater than in the other treatments. It seems unlikely however, that the increases in herbage copper content with slurry, slurry extract or $CuSO_4$ could have been influenced by the quantities of the major nutrients added in view of the fact that the copper concentrations in the control herbage receiving N, P and K at rates R1 and R2 did not differ significantly.

The uptake of trace elements by plant roots would appear to be related to the activity or effective concentration of the elements at the root surface (Baker, 1971, 1973). Thus, the addition of copper in solution to that already present in the soil solution might be expected to result in increased copper uptake by crops growing on the soil. The present investigation has shown that copper in the aqueous phase of slurry may increase the level of acetic acid extractable copper in the soil, the fraction which is thought to be adsorbed on inorganic sites (Mitchell, et al, 1957a,b) and readily available to plants through desorption into the soil solution. Furthermore, it has been shown that copper in this slurry fraction has the potential to contribute to the enhanced copper concentrations observed in herbage following slurry application under field conditions. This finding would support the suggestion that dilution of slurry before application may result in greater copper concentrations than undiluted slurry due to a greater transfer of slurry copper to plant root systems. This dilution effect would obviously depend on the initial D.M. content of the slurry and indeed it was observed (Chapter 3, this thesis) that dilution of slurry to D.M. contents lower than approximately 5 g/100 g resulted in little, if any, further increase in herbage copper content.

In view of the equilibrium between the various soil copper fractions (Viets, 1962) and the bonding strength of adsorption sites on soil materials such as iron and manganese oxides and organic matter (McLaren and Crawford, 1973b) it is unlikely that copper added to soil solution would remain in this fraction. Uptake of slurry copper might therefore be influenced by the removal of this copper from soil solution due to adsorption processes. The residual effect of copper sulphate on herbage copper concentrations has been found to be small (Reith and

Mitchell, 1964). In the present investigation, copper added to the soil in slurry extract did not affect herbage copper concentrations at the second harvest. This decrease in availability might therefore have contributed to the decrease in concentration of copper in herbage grown from grazing to ear-emergence stage.

In contrast to the findings of the present investigation, Mutlak (1974) reported that the effluent from an oxidation ditch, for the biological treatment of pig slurry, did not produce any consistent effect on herbage copper content when applied to soil in a pot experiment; the effluent contained 1 mg Cu/l and was applied at rates greater than those used in the present study. Mutlak (1974) also reported increases in herbage copper content when effluent from an anaerobic slurry lagoon was applied to soil, while Kneale and Smith (1977) considered that undefined chemical changes occurring in an anaerobic storage tank had rendered the copper unavailable when this material failed to elevate the copper content of a ryegrass sward.

These conflicting reports would suggest that further research is necessary to elucidate the forms of copper present in untreated slurry stored under different conditions and in the effluent from slurry treatment units; such work should also be accompanied by studies of the availability to crops, of the copper present in these materials.

CHAPTER 6.

A PRELIMINARY INVESTIGATION INTO THE RATE OF DECOMPOSITION IN SOIL, OF FAECES OF PIGS FED A DIET CONTAINING NO ADDED COPPER OR WITH COPPER ADDED AS A GROWTH PROMOTER.

6.01. Introduction.

The biodegradation of animal wastes is important with regard to its decomposition in soil with concomitant release of plant nutrients and its biological treatment in systems now obligatory (Hobson and Robertson, 1977) on many farms where, due to intensity of production, sufficient land is not available for utilisation of the wastes as fertiliser.

Of primary importance in any consideration of the decomposition of biological materials, including animal excreta, is the presence or absence of substances which inhibit metabolism in microorganisms and the chemical form of the inhibitors, if present. The biocidal properties of copper are well known (reviews by Beran, 1965 and Coombs, 1965), Cu having been used as a fungicide, bacteriocide and molluscicide for almost a century now. In view of the high concentrations of copper in pig slurry and the known inhibitory effect of copper on aerobic bacteria, Robinson et al (1971) investigated its effect on Chemical Oxidation Demand (C.O.D.). In these studies addition of 50 to 500 mg Cu/l inhibited reduction of C.O.D.

Bacterial and plant polysaccharides have been shown to form complexes with metal ions (Mortensen, 1963). The rate and extent of decomposition in soil of prepared polysaccharide-metal complexes has been reported to be lower than that for the metal-free polysaccharides (Martin et al, 1966); copper apparently reduced decomposition to the greatest degree. In view of the findings that the fine particulate and colloidal fraction of solids in copper-rich pig slurries may contain in excess of 2,000 mg Cu/kg D.M. (Chapter 4, this thesis), a study was conducted to examine the rate of decomposition in soil of this fraction isolated from the faeces of pigs fed rations with and without supplementary copper. Oxygen uptake by moist soil containing added faeces was used to assess the activity of soil microorganisms (Webley, 1947), which would reflect the rate of decomposition of slurry solids plus the organic matter native to the soil.

6.02. Collection and preparation of pig faeces.

Six pigs ranging in liveweight from 40 to 46 kg were housed in individual metabolism crates and offered 1.5 kg/day of a ration consisting of:

Barley	840 g/kg
Soya bean meal	100 g/kg
White fish meal	60 g/kg
Calcium carbonate	6 g/kg
Dicalcium phosphate	6 g/kg

A mineral/vitamin supplement (Parkhill, No. 2, Isaac Spencer & Co. Ltd., Aberdeen) was added to this basal ration to give a final copper concentration of 250 mg Cu/kg D.M. A supplement of the same composition except for the omission of copper was added to the basal ration, giving a second ration with a total copper content of 28 mg/kg D.M.

Three pigs were fed the copper supplemented ration and three received the ration containing no added copper; the rations were offered ad libitum. After a period of 14 days the faeces from each pig were collected in polythene bins each day, for four days. The faeces produced by each pig over the four days were combined and the fine particulate plus colloidal material separated using the fractionation scheme described for slurry solids (Chapter 4, this thesis).

The separated solids were dried to constant weight at 80°C and milled to pass a 500 micron mesh screen.

6.03. Determination of oxygen uptake by soil alone and soil containing added low or high copper faecal solids.

The soil used in this investigation was a topsoil of the Darvel series, Darvel Association (The Soils of the Bush Estates, Midlothian, 1969). The soil was air dried and the fraction passing a 500 micron mesh screen used for the oxygen uptake studies.

The manometric method described by Webley (1947) was used to determine oxygen uptake by soil. The isolated faecal fractions from pigs receiving rations of the same copper content were combined giving a single sample of high copper and a single sample of low copper faecal solids.

Aliquots, (5 g) from the high and the low copper solids were mixed with 100 g of soil and 4 g of the amended soils weighed out and placed in Warburg flasks. There were 6 replicates for soil containing high copper solids and 6 for soil containing the low copper solids.

A predetermined amount of distilled water was added to bring the soil in each flask to 50% saturation and the flasks weighed. The soils were incubated in a water bath at 25°C without shaking, the side arm of each flask remaining open to the atmosphere except when oxygen uptake was being measured over a 5 hour period daily; oxygen uptake was recorded over a period of 28 days.

Copper in the faeces fractions was extracted using 0.02 M EDTA and 1 N HCl and copper in soil by 0.02 M EDTA; the extraction procedures and method for determining the concentration of copper in the extracts are given in Appendix II. The concentration of copper in faeces fractions was determined as described for slurry solids (Appendix IV).

6.04. Results.

The concentration of copper in the solids fractions derived from the faeces of pigs receiving rations containing 250 mg Cu/kg D.M. ranged from 1892 to 2505 mg Cu/kg D.M., the concentration in the bulked material being 2278 mg/kg D.M. The copper concentrations in faeces fractions from individual pigs receiving the low copper diet ranged from 98 to 124 mg/kg D.M., with a concentration of 118 mg/kg D.M. in the bulked material.

The small particulate plus colloidal solids fractions isolated from the faeces of pigs receiving the high and low copper diets were dark grey/black in colour and changed to a light straw colour during extraction with HCl or EDTA. Hydrochloric acid extracted 98.2 and 97.5% of the total copper present in the bulked samples of the high and low copper fractions respectively; EDTA extractability of copper in these fractions was 75.6 and 79.2% respectively.

Oxygen uptake by soil containing the high copper faeces fraction did not differ significantly from that of soil containing the low copper fraction, the mean total uptakes (± S.E.) for the 28 day incubation period being 13.8 ± 0.42 and 13.2 ± 0.36 cm^3 O_2 respectively at 25°C.

6.05. Discussion.

The extractability of copper in the faeces fractions using HCl and EDTA would suggest that the copper was not present as the sulphide, but adsorbed on inorganic or organic sites as was found for dried slurry solids (Chapter 4, this thesis). Although the faeces fraction from pigs fed the copper supplemented diet added a total of 114 mg Cu/kg to the soil incubated, oxygen uptake by soil microorganisms was not affected. Similarly, Cornfield et al (1976) found no evidence for a reduction in microbial activity in soils containing 123 mg Cu/kg during studies on carbon dioxide evolution and nitrogen mineralisation by soils following addition of sewage sludges. In earlier studies (Premi and Cornfield, 1969) reported that soil microbial activity was enhanced in soil containing 1000 mg of added copper/kg.

At present, the tolerance of the various soil organisms to heavy metals is unknown. The findings of Tyler (1975) that nitrogen mineralisation in soils may be adversely affected at soil copper concentrations greater than 50 mg Cu/kg would suggest that N-mineralisation may be more sensitive to high concentrations of heavy metals in soils than in carbon mineralisation. Since the phytotoxicity of heavy metals in soils has been shown to be greater when the elements are added as inorganic salts than as sewage sludge, organic matter added with the metals may well have an ameliorating effect.

Although the present study, which is no more than preliminary in nature, has shown that microbial activity in soils was not affected by addition of high copper faecal material, long term studies should be carried out under field conditions to assess the effect of frequent slurry copper additions to soil on microbiological processes as the copper accumulates in the soil.

CHAPTER 7.

DETERMINATION OF THE AVAILABILITY TO SHEEP OF COPPER IN PIG SLURRY AND IN HERBAGE FROM SLURRY TREATED LAND

7.01. Introduction

The inclusion of copper as a growth stimulant at levels of up to 250 mg Cu/kg in the rations for fattening pigs results in the production of manures or slurries containing high concentrations of this element. Slurry used in the investigations reported in this thesis was obtained from the E.S.C.A. pig unit where the rations contained 200 mg supplementary copper/kg, a level of supplementation used in most fattening rations in Scotland (Miller, 1974). The slurry, containing from 529 to 779 mg Cu/kg D.M., when applied to grassland, markedly increased the concentration of copper in perennial ryegrass and white clover; maximum copper concentrations recorded in separate experiments were 24.4 mg Cu/kg D.M. in the ryegrass and 24.8 mg Cu/kg D.M. in the clover. Copper concentrations up to 103 mg Cu/kg D.M. have been reported in herbage following heavy applications of pig slurry to growing swards (Batey et al, 1972; Lawrence and Gibbs, 1973; Kneale and Howell, 1974). It has also been shown (Batey et al, 1972) that slurry solids adhering to the surface of herbage, especially after application to long grass, may result in copper concentrations of up to 338 mg Cu/kg D.M. in unwashed herbage.

According to Underwood (1971), sheep and milk-fed calves are more susceptible to copper poisoning than other farm livestock, mature cattle, pigs and poultry tolerating high intakes of this element for long periods of time. Since copper poisoning has occurred in sheep fed rations containing 15 mg Cu/kg D.M. or less (Suttle, 1968; Buck, 1970), concern has been expressed (Batey et al, 1972) regarding the potential toxicity hazard to sheep consuming fresh or conserved herbage from land used for disposal of copper-rich pig slurries. At the time the investigations reported in this thesis were carried out, reports regarding the importance of slurry copper as a hazard to sheep were conflicting. Copper poisoning was reported to have occurred in sheep fed hay contaminated with pig slurry (Feenstra and Van Ulsen, 1973). In contrast, Woodside (1973) concluded that there is little risk of copper poisoning in sheep grazing slurry treated pasture, even where the herbage is visibly contaminated with copper-rich slurry.

In view of the doubt as to whether or not pig slurry disposal on pasture constituted a hazard to sheep, it was considered important to obtain information as to the availability to sheep of copper in herbage containing enhanced levels of this element resulting from additions of slurry copper to pasture. It was also considered necessary to assess the availability of copper in pig slurry itself since slurry copper may be ingested by sheep in the following ways:

(i) as a result of slurry solids adhering to herbage after spreading.

(ii) as a result of ingestion of surface soil containing slurry solids. Healy and Ludwig (1965) have reported that soil may at times contribute up to 20% of the dry matter intake of grazing sheep.

(iii) as a result of the deliberate addition of dried pig wastes to rations for ruminant livestock (Bhattacharya and Taylor: review, 1975).

The objectives of this investigation were therefore to assess:-

(i) the availability to sheep of copper in herbage grown on slurry treated land and to compare it with the availability of copper in herbage from untreated land.

(ii) the availability to sheep of slurry copper ingested directly.

The availability of copper in copper sulphate was also determined as this inorganic form of copper is readily utilised by livestock (Underwood, 1971).

7.02. Selection of a method for the determination of the biological availability of copper to sheep.

Conventional balance techniques for measurement of the difference between dietary intake of an element and the amounts excreted in the urine, faeces and sweat plus losses in hair or wool and skin are not suited to the determination of copper availability as only a small proportion of this element ingested (10% or less) is actually absorbed and hence differences in absorption may be completely masked by inaccuracies in determining the losses of the element from the body (Mills and Williams, 1971).

The oral repletion techniques used in the past have simply measured the relative efficiency of copper utilisation for particular body functions such as synthesis of the copper containing enzyme, caeruloplasmin (Kirchgessner and Grassman, 1970) or haemoglobin synthesis (Mills, 1957) rather than the efficiency with which the element is removed from the diet.

Other workers have frequently assessed the efficiency of dietary copper utilisation from changes occurring in liver copper stores (Dick, 1953; Van der Veen and Keener, 1964; Hartmans and Van der Grift, 1965; Hogan, Ris and Hutchinson, 1966). Substantial errors may, however, arise in this method owing to variation in copper concentration from one part of the liver to another (Hogan, Money and Walker, 1971). Inaccuracies can therefore occur in estimating changes in total liver copper content from liver biopsy samples and additional errors may be introduced in predicting total liver weight from animal liveweight.

The method recently published by Suttle, 1974 was selected for the determination of copper availability since it provided greater sensitivity, required for the comparison of the availabilities of copper from different sources than any of the techniques described above. The method is based on the assumption that infusion of copper into the jugular vein adequately simulates the post-absorptive movement of dietary copper from the cells of the gut wall into the blood stream.

Sheep are initially depleted of copper by feeding a semi-synthetic diet (Suttle and Field, 1968) containing approximately 1 mg Cu/kg D.M. Repletion of the sheep is then carried out by slow intravenous infusion of a copper salt over a period of up to 28 days. From the repletion study, a regression equation may then be derived for the sheep relating their response in plasma copper concentration to the rate of infusion of copper. These sheep are again made hypocupraemic and fed the ration under test or the semi-synthetic diet to which has been added copper in the form being investigated. From the response in plasma copper concentration the availability of copper in the ration, expressed as grammes of Cu absorbed into the blood stream/gramme of Cu in the diet may be calculated using the equation relating plasma copper response to rate of intravenous infusion which is assumed to simulate the rate of passage of dietary copper into the blood stream.

The usefulness of this technique is limited by the sigmoid nature of the copper intake versus plasma copper response curve due possibly to changes in either the efficiency of copper absorption or in the metabolism of absorbed copper at extreme copper inputs. The concentration of copper in the diet fed may however be adjusted using the semisynthetic diet so that extreme copper intakes, on the high side, may be avoided. Suttle (1974) has shown that this method is sensitive enough to detect significant differences between treatments differing by only 0.045 mg Cu/day in the available copper they provided.

7.03. Production of herbage and preparation of pig slurry for copper availability determinations.

The site for production of herbage was located on an imperfectly draining clay-loam soil of the Winton series, Rowanhill Association on the E.S.C.A. farms, Bush Estates, Midlothian. The characteristics of the soil are given in Appendix I. Two adjacent plots each measuring 48 x 25 metres were marked out on a uniform area of an established perennial ryegrass sward on the site. Each plot was divided into 3 sub-plots measuring 16 x 25 metres. Mineral fertiliser (S.A.I., No. 2) had been applied to the sward 2 weeks before this investigation commenced and supplied 60 kg N, 14 kg P and 25 kg K/ha.

At weekly intervals, commencing in May 1974, herbage on one sub-plot from each of the two main plots was cut back close to ground level using a motor-mower. Pig slurry was then applied each week to one of the trimmed sub-plots, the other being left untreated. This procedure of applying slurry over a period of three weeks was adopted in an attempt to minimise the range in maturity of herbage offered to sheep over a 3 to 4 week repletion period, since Fleming and Murphy (1968) have reported large differences in the copper content of grasses in relation to maturity.

The copper-rich pig slurry which was obtained from under-floor storage tanks at the E.S.C.A. pig fattening unit, Easter Howgate, was applied at 70,000 l/ha, a rate similar to that shown to markedly increase herbage copper concentrations (Chapter 2, this thesis). The slurry, which was produced by pigs fed rations containing supplementary copper at a level of 150 mg/kg, contained 22 g D.M./100 g and 429 mg Cu/kg D.M.

A 15 kg sample of the slurry was drawn from the tanker at each application and retained for analysis or incorporation into a semi-synthetic diet used in the oral repletion of sheep as described in section 7.05. The slurry sample retained was dried at 80°C for 48 hours and ground in a hammer mill to pass a 1 mm screen.

7.04. Animals and housing.

The availability of copper in (i) herbage grown on slurry treated or untreated land and (ii) copper-rich pig slurry and copper sulphate was determined in two experiments, A and B, using in each experiment 12 Scottish Blackface ewes which were 2 years old. In a previous uniformity trial (Suttle, 1974) these sheep had been made hypocupraemic and the response in plasma copper concentration (y, ml/l) following intravenous infusion of a copper sulphate solution found to be related to the rate of infusion (x, mg Cu/day) by the equation $y = 2.135x - 0.16$.

In the present investigations the sheep were depleted of copper by feeding a semi-synthetic diet formulated by Suttle and Field (1968) and containing less than 1.2 mg Cu/kg D.M. When plasma copper concentrations had fallen below 0.3 ml/l (range 0.13 to 0.28 mg/l) the sheep were ranked in order of plasma copper response to oral repletion as obtained by Suttle (1974); the ranking list was divided serially into blocks of 6 and treatments allocated to ewes at random within blocks. Two groups of 6 ewes were used in Experiment A to assess the availability of copper in herbage and a further two groups in Experiment B to compare the availabilities of copper in pig slurry with that in copper sulphate. The experiments were of a randomised block design and lasted 28 days.

The sheep were individually housed for the duration of the experiments in pens described by Suttle and Field (1968).

7.05. Oral repletion procedure.

The hypocupraemic sheep were repleted with copper over a period of 28 days by feeding either fresh herbage (Experiment A) or a semi-synthetic (S.S.) diet (Suttle and Field, 1968) to which was added pig slurry or copper sulphate. Each animal received 0.8 kg air dry S.S. diet/day or 4 kg fresh herbage/day; deionised water was always freely available. The oral repletion treatments were:-

EXPERIMENT A

Group 1 4 kg fresh weight of untreated herbage/day.

Group 2 4 kg fresh weight of slurry treated herbage/day.

EXPERIMENT B

Group 1 S.S. diet containing 8 mg supplementary copper/kg D.M. added as $CuSO_4.5H_2O$.

Group 2 S.S. diet containing dried pig slurry; 19.8 g slurry D.M./kg diet providing 8.5 g supplementary copper/kg D.M. in the diet.

The S.S. diet contained 0.9 mg Cu/kg D.M. On commencement of repletion, intakes of herbage were increased to the maximum of 4 kg/day in daily increments of 1 kg, the allowance of copper deficient S.S. diet being correspondingly reduced by 0.2 kg/day from 0.8 kg to 0.0 kg/day.

Initially, slurry treated or untreated herbage was cut every two days, the first cut being taken 30 days after the first slurry application with the sward at ear-emergence stage. However, in order to minimise the range in maturity of the grass offered to the sheep from day 18, herbage for the final 10 days of the experiment was cut and stored in polythene sacs at $-15^{\circ}C$. Portions were removed every two days to be thawed before feeding. Samples of herbage fed on days 1 to 8 were combined and copper analysis carried out on the bulk sample. However, since D.M. content was thought to be changing rapidly, individual samples from days 10 to 28 were retained for analysis.

A blood sample (5 ml) was taken from individual sheep once per week by jugular venipuncture using a stainless steel needle; the blood was collected in 10 ml acid-washed centrifuge tubes for copper determination. The availability of dietary copper was estimated from the increase in plasma copper concentration from the initial value to the value at day 21 using the equation $y = 2.135x - 0.16$ relating plasma copper response (y, mg/l) to intravenous infusion rate (x, mg/day) derived for sheep by Suttle (1974) in a separate study. Since the above equation was derived using sheep maintained on a low copper diet providing

0.044 mg available copper (Suttle, 1974) this quantity was added to that estimated from the equation to have been absorbed daily (y, mg/day).

7.06. Analytical methods.

Herbage and diet samples (200 g) were dried at 80°C for 24 hours and hammer milled to pass a 1 mm screen. A 1 to 2 g aliquot of the milled S.S. diets was digested with a concentrated nitric acid/perchloric acid mixture (20:1 v/v) in acid-washed pyrex boiling tubes. The digests were taken to dryness and the residues taken up in 10 ml of 1.5 N HCl. A blank was carried through this procedure and duplicate samples of the S.S. diets were digested. The concentrations of copper in the digests were determined by atomic absorption spectrophotometry (Varian Tectron A.A.5) using an air-acetylene flame and standards containing 0.5 to 2.0 mg Cu/l in 1.5 N HCl.

The concentration of Cu, Zn, Mn and Fe in herbage and slurry dry matter were determined by the methods given in Appendix IV. The concentration of EDTA-extractable copper in soil samples was determined by the procedure given in Appendix II.

The extractability of copper in dried slurry was determined by extracting 2 g samples of slurry dry matter with 100 ml of 1 N HCl or 0.02 M EDTA solution (di-sodium salt, pH 4.2); the extraction time was 4 hours and the concentration of copper in the extract determined as for soils extracts.

Blood samples were centrifuged at 3000 x g for 15 minutes and the plasma drawn off. Plasma was diluted with an equal volume of glass distilled water containing HCl giving a normality of 0.001 N. The concentration of copper in the diluted plasma was determined by atomic absorption spectrophotometry using an air/acetylene flame and copper standards ranging in concentration from 0.1 to 0.5 mg/l in 0.001 N HCl.

All glassware used in this investigation was acid washed to remove traces of copper; the glassware was steeped for 24 hours in 6 N HNO_3 (Analar grade), washed with glass distilled water and dried before use.

In each experiment, the standard error of the difference between treatment means was based on a pooled estimate of variance from analyses of variance carried out on plasma copper response and on availability data.

7.07. Results.

The concentrations of Cu, Zn, Mn and Fe in slurry used in this investigation were 429, 415, 261 and 4487 mg/kg D.M. respectively. The mean concentration of EDTA-extractable copper in the 0 to 7.5 cm layer of soil from the untreated sub-plots was 5.6 ± 0.06 (S.E.) mg/kg compared with 7.7 ± 0.18 mg/kg soil for the slurry treated area; the difference between means being significant (P<0.001). The extractability of copper in the slurry solids applied to the soil and fed to the sheep was found to be 78.3% using EDTA and 98.8% using HCl.

The copper and D.M. contents of herbage from the slurry treated and untreated areas are shown in Figure 7.1. By day 10 of the repletion period (40 days after slurry application to the first sub-plot treated) the dry matter content of treated and untreated herbage was increasing rapidly and from day 13 onwards the D.M. content of the untreated herbage was greater than that from the slurry treated area. Although from day 18 onwards herbage was stored deep frozen, the D.M. content appeared to decrease.

The effect of slurry application on herbage copper content was small, the difference between treated and untreated herbage falling from 2.9 mg/kg D.M. for combined samples of the material harvested between days 1 and 10 to 1.9 mg/kg D.M. by day 28 of the repletion period.

The average daily copper intakes for sheep in Experiment A were 6.2 and 7.0 mg Cu/day for those receiving herbage from untreated and slurry treated areas respectively, while sheep given the S.S. diet had daily copper intakes of 6.4 mg Cu from $CuSO_4$ and 6.8 mg Cu from pig slurry.

The effects on plasma copper concentration of repleting the initially hypocupraemic ewes with copper from the different sources are shown in Figure 7.2. and the responses in plasma copper of individual ewes after 7, 14, 21 and 28 days repletion tabulated in Appendix VIII. In each treatment group in each experiment, copper concentrations increased linearly with time following an initial lag. Ewes given herbage from the slurry

FIGURE 7.1.

Copper (●, o) and dry matter (■,□) contents of slurry treated (solid symbols) and untreated (open symbols) herbage.

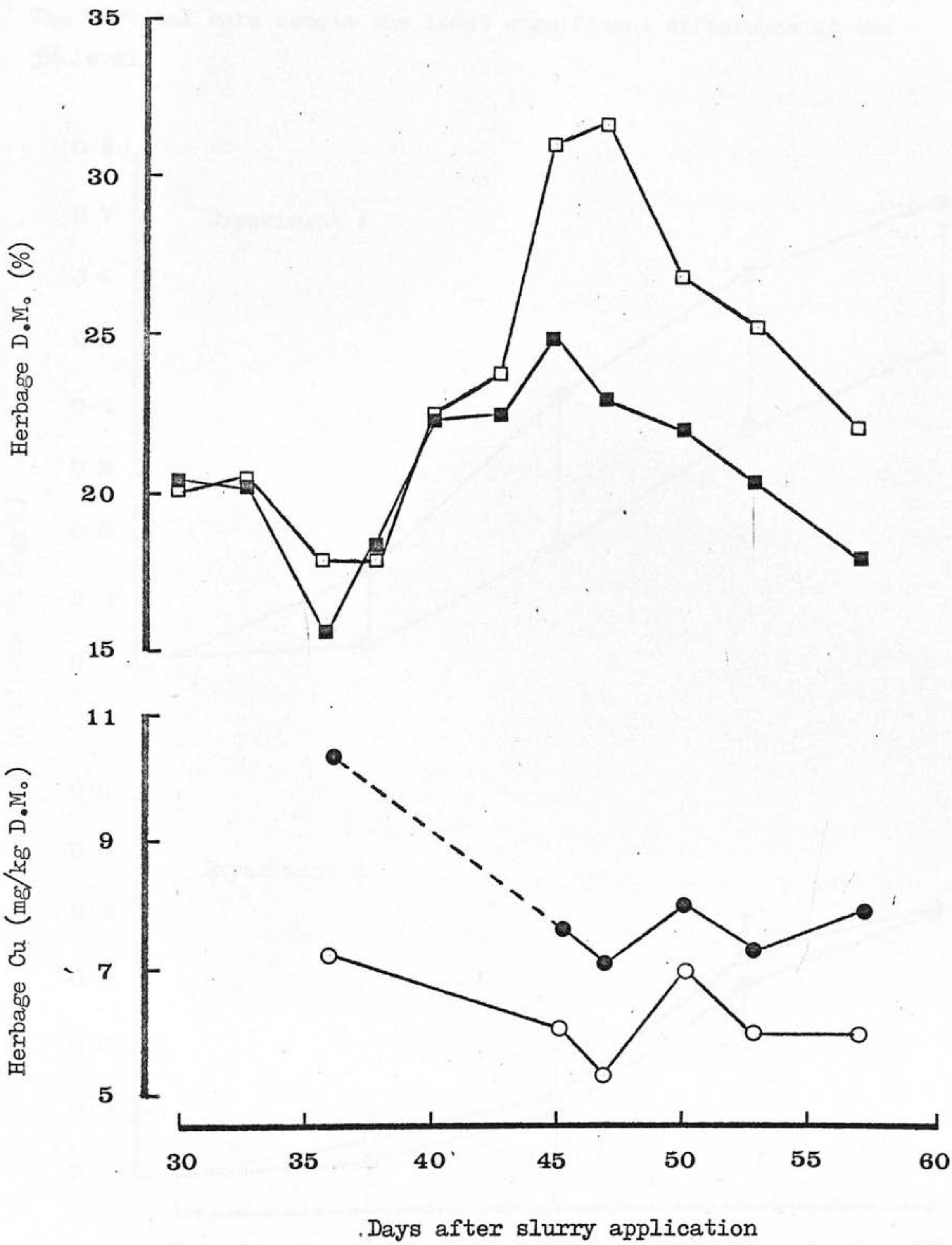
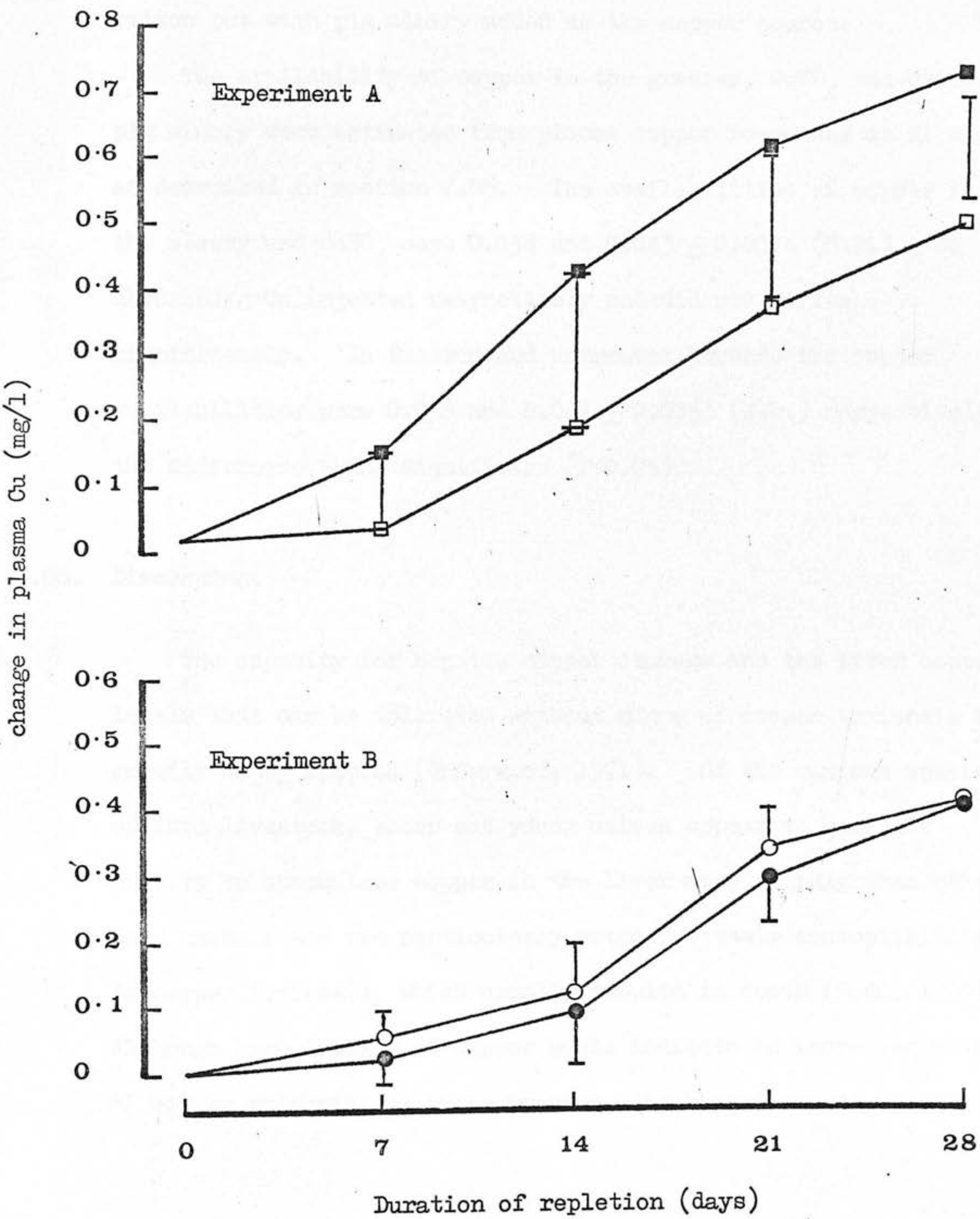


FIGURE 7.2.

Changes in plasma copper of initially hypocupraemic ewes repleted with: Experiment A, slurry treated (■) or untreated (□) herbage; Experiment B, dried pig slurry (●) or $CuSO_4$ (○). The vertical bars denote the least significant difference at the 5% level.



treated sward repleted more rapidly than those given herbage from the untreated area, the mean changes (response) in plasma copper concentrations for the two groups differing significantly ($P<0.05$) at each sampling. The mean response in plasma copper of ewes fed the S.S. diet containing $CuSO_4$ was not significantly different at any time to that of ewes receiving the same basal ration but with pig slurry added as the copper source.

The availability of copper in the grasses, $CuSO_4$ and dried pig slurry were estimated from plasma copper responses at 21 days as described in section 7.05. The availabilities of copper in the slurry and $CuSO_4$ were 0.038 and 0.043 $\pm$ 0.0076 (S.E.) g Cu absorbed/g Cu ingested respectively and did not differ significantly. In treated and untreated herbage the copper availabilities were 0.058 and 0.047 $\pm$ 0.0033 (S.E.) respectively, the difference being significant ($P<0.05$).

7.08. Discussion.

The capacity for hepatic copper storage and the liver copper levels that can be tolerated without signs of copper toxicosis vary greatly among species (Underwood, 1971). Of the various species of farm livestock, sheep and young calves appear to have the ability to accumulate copper in the liver more rapidly than other farm animals and are particularly noted for their susceptibility to copper toxicosis, which usually results in death (Todd, 1969). Although high intakes of copper would indicate an increased risk of copper poisoning in these species, the hazard may depend as much upon the availability to the animal of the forms of copper in the ration as on the total intake of the element. Copper in copper sulphide, for instance, is almost completely unavailable to both ruminants and monogastric animals (Underwood, 1971) and therefore of low toxicity. Since Robel and Ross (1975) have suggested that the copper in pig slurry may exist in this form, slurry copper ingested by sheep should therefore present little risk with regard to excessive hepatic accumulation of this element. Although the studies reported by Woodside (1973) would support this suggestion, Feenstra and Van Ulsen (1973) have reported cases of copper poisoning in sheep given hay contaminated with solids from copper-rich pig slurry.

The results of the present investigation have shown that slurry copper in rations may rapidly replete copper deficient sheep and that the availability of this copper may be similar to inorganic copper as copper sulphate, which is reputed to be one of the most available forms of this element to animals (Underwood, 1971). Although further studies on the true availability of slurry copper have not as yet been reported, the findings of recent investigations into the retention of copper by sheep fed rations containing copper-rich pig slurry would indicate a high availability of this form of copper. Dalgarno and Mills (1975) reported that the copper content in liver tissue of sheep rapidly increased in response to the inclusion of 1 or 2% slurry solids in the diet, liver copper concentrations rising from 12 to 474 mg/kg D.M. over a period of 123 days at the latter level of inclusion. Dried pig faeces, containing 613 mg Cu/kg D.M. have been added to sheep rations at the much higher levels of 15 and 30% of the ration D.M. (Hendrosoekarjo and Pearce, 1978) and shown to result not only in hepatic accumulation of copper but also to cause liver necrosis in varying degrees. It is interesting to note that the inclusion of dried animal excreta at levels of up to 30% in rations has been shown to be palatable to ruminants and economically acceptable in terms of livestock performance; for a review of this subject see Bhattacharya and Taylor (1975). Dried pig slurry containing 623 mg Cu/kg D.M. and constituting 30% of the D.M. in a ration as described by Hendrosoekarjo and Pearce (1978) would increase the copper content of the ration by 187 mg/kg D.M., a dietary copper concentration undesirable even for mature ruminants, which are considerably more tolerant (Underwood, 1971) of high copper intakes than sheep.

In the present investigation, the increase of 2.9 mg Cu/kg D.M. in herbage at ear-emergence stage following the single application of pig slurry was similar to the increases reported earlier (Chapter 3, this thesis) for ryegrass sampled at the same stage of maturity. This increase was small, however, in comparison to those observed in less mature herbage sampled at grazing stage. The greater response in plasma copper concentration promoted by herbage from the slurry treated sward may partly be attributable to its lower D.M. content since Hartmans and Bosman (1969) have reported that in cattle, copper retention may be greater with mature than with young herbage. Copper in herbage from both the treated and untreated swards was found to be as available to sheep as copper in $CuSO_4$.

Uncertainty as to the extent to which the copper of pig slurry is absorbed by plants or is merely retained as residues on leaf surfaces has been regarded as an important question requiring an answer before the potential hazard created by copper-rich slurry can be assessed. The investigations reported earlier (this thesis, chapter 3) have shown that slurry copper applied to a ryegrass sward may increase the concentration of copper in the herbage to levels in excess of 20 mg Cu/kg D.M. in the absence of surface contamination. Since copper poisoning has been reported in sheep fed rations containing 15 mg Cu/kg D.M. (Suttle, 1968; Adamson et al, 1969; Buck, 1970) the finding that copper at grazing stage in herbage from slurry treated land is comparable in availability to that in copper sulphate would suggest that such herbage may represent a hazard to grazing sheep, particularly in situations where there is a high proportion of clover in the sward. Copper concentrations of slurry treated herbage were, however, found to decrease towards those of untreated herbage when sampled at a later stage of maturity. In addition, the residual effect of slurry Cu on herbage Cu level was very small in the second crop after a slurry application. Thus, unless slurry is applied regularly to pasture an increased copper intake would not be maintained in grazing livestock.

The greatest hazard to sheep or young calves would therefore appear to be associated with the ingestion of slurry solids. Reference has already been made to the quantities of slurry copper which may be found adhering to the surface of herbage following slurry application to a growing sward (Batey et al, 1972). Furthermore, Kneale and Smith (1977) have reported changes in a number of biochemical parameters, such as increases in the activity of the enzyme glutamate oxaloacetate transaminase (S.G.O.T.) in blood serum, indicating liver damage in sheep grazing pasture heavily dressed with copper-rich slurry.

At present it is not clear to what extent slurry solids remaining on the soil surface after spreading are picked up by grazing livestock. Field and Purves (1964) have shown that appreciable quantities of soil may be ingested by sheep during the winter months when there is little grass growth and Healy and Ludwig (1965) have reported that soil may contribute from 0.5 to 20% of the D.M. intake of grazing sheep. Since concentrations of 50 to 60 mg EDTA-extractable slurry copper/kg were found in the surface 2.5 cm of soil plus slurry solids (this thesis, chapter 3), this material would increase the concentration of copper in the diet by 10 to 12 mg/kg D.M. if 20% of the dry matter intake consisted of this material. These increases in copper concentration may well be conservative as it is unlikely that soil from a depth of 2.5 cm would be picked up and hence there would be less dilution, by soil, of the surface slurry solids ingested. Dalgarno and Mills (1975) have reported that the copper content of slurry residues on the soil surface may be 70 times greater than that of growing herbage. In addition, these workers found that slurry residues on the soil surface may be picked up by forage harvesters in the process of cutting the crop for silage or dried grass production, particularly under dry conditions. The accumulation of copper-rich slurry residues on the soil surface therefore clearly represents a potential source of copper for both grazing and housed livestock and the resultant increased copper intakes are likely to be very variable, depending on the level of soil ingestion or the weather conditions prevailing at the time the herbage is cut by forage harvester.

Although the present investigations have shown that the availability of copper in slurry treated herbage and in dried slurry solids is comparable to that of copper sulphate, it is difficult to predict the length of time required for the elevated copper intakes to bring about accumulation of this element in the livers of sheep to such an extent that symptoms of copper poisoning appear. The length of time required for the development of symptoms of toxicity would depend on the initial concentration of copper in the liver of the animal, the level of intake of slurry copper, the availability of the copper and the presence or absence of factors in the diet such as molybdenum and sulphur (Suttle and MacLauchlan, 1976) which may influence copper availability and retention.

Kneale and Smith (1977) reported increased liver copper concentrations and changes in enzyme levels indicating liver damage in sheep and lambs grazed for 6 months on pasture where the concentration of copper in unwashed herbage ranged from 20 to 175 mg Cu/kg D.M. following winter application of 250,000 l of copper-rich slurry/ha. These workers concluded that some of the sheep were close to death from copper poisoning. This conclusion must however be questioned as the liver copper levels reported, 111.8 and 544 mg Cu/kg D.M. for lambs and sheep respectively are very unlikely to cause a haemolytic crisis, which usually occurs only when liver copper concentrations exceed 1,000 mg Cu/kg D.M. (Todd, 1969); the liver copper levels for control lambs and ewes in this study were 25.2 and 339 mg Cu/kg liver D.M. respectively. Thus, although liver damage in these sheep was evident even on visual examination, it seems unlikely that copper accumulation was the cause. From a study of retention of copper in the liver by sheep fed slurry solids containing 474 mg Cu/kg D.M. at a level of 2% in the ration, Dalgarno and Mills (1975) estimated that a significant risk of copper poisoning would exist if this level of intake continued for more than 250 days.

Copper-rich pig slurry produced at the E.S.C.A. fattening unit was found to contain zinc and iron at concentrations ranging from 450 to 700 and 7,500 to 12,000 mg/kg D.M. respectively (Appendix VI). Elevated intakes of these elements have been shown (zinc, Van Campen and Scaife, 1967; iron, Campbell et al, 1974) to reduce retention of copper in the liver. To obtain a significant reduction in accumulation of copper in the liver it would appear from the work of Bremner et al (1976) that pig slurry would need to be present at a level of 50 to 100% in the diet to provide sufficient zinc; similar amounts of dried slurry would be required for iron to affect copper accumulation judging from the work of Campbell et al (1974).

In contrast to the conclusions of Dalgarno and Mills (1975), Suttle and Price, 1976, and Kneale and Smith (1977), Woodside (1973) concluded that there is little risk of copper poisoning in sheep grazing slurry treated land. These apparently conflicting observations may well be a reflection of differences in the conditions to which pig slurry has been exposed between voiding by pigs and ingestion by sheep. Dalgarno and Mills (1975) have suggested that when slurry is held under anaerobic conditions, the accompanying accumulation of hydrogen sulphide (McAllister, 1973) may have the beneficial effect of rendering any copper unavailable to sheep by the formation of insoluble cupric sulphide. In support of this suggestion, Kneale and Smith (1977) found that slurry from an effluent treatment plant failed to elevate herbage copper levels and considered that undefined changes taking place in an anaerobic storage tank had rendered the slurry copper unavailable to herbage. The findings (chapter 4, this thesis) that copper sulphide is rapidly oxidised to the sulphate under moist aerobic conditions, conditions which would exist at the time of spreading of slurry on pasture, would tend to argue against this sulphide theory.

While the present investigations have shown that copper in slurry treated herbage and dried pig slurry is available to sheep, uncertainty as to the form in which copper occurs in untreated slurry when stored under different conditions or in the products of biological treatment systems to reduce B.O.D., would suggest that further research is required before predictions as to availability of slurry copper to animals and indeed to crops may be made with confidence.

CHAPTER 8.

GENERAL DISCUSSION

The addition of copper sulphate to pig fattening rations to give dietary copper concentrations four to five times greater than the 50 mg/kg required by the pig (A.R.C., 1967) has been shown, in a large number of studies, in different countries, to result in increased growth rate and improved efficiency of feed conversion (reviewed; Braude, 1965, 1975). The mode of action of copper in producing this effect is as yet unclear and in a number of instances negative responses in terms of growth rate have been obtained. However, over all the studies reviewed by Braude, supplementation of pig fattening rations with up to 250 mg Cu/kg D.M. resulted in an average improvement in feed conversion efficiency of 7.4%, associated with an increase in growth rate of 9.1%. Responses of this magnitude are not only impressive, but economically significant in pig farming.

The adoption of copper supplementation of pig fattening rations as a means of optimising production has led to a market situation in which pig producers have become economically dependent upon the growth promoting effects of the supplemented rations. Although legislation has been passed within the European Economic Community (E.E.C. Directive, 1972) stipulating that the concentration of copper in animal feedstuffs should not exceed 125 mg Cu/kg D.M., this Directive has not as yet been implemented in Great Britain and copper continues to be added to pig rations at near optimum levels for growth promotion. The level of copper supplementation most commonly used has been reported (Miller, 1974) to be 200 mg/kg of ration; reduction of the dietary concentration of copper to 125 mg/kg D.M. would, according to Braude (1965), halve the growth promotional effect.

Since over 90% of the copper ingested by pigs is excreted again in the faeces, concern has been expressed with regard to the consequences of disposing of these high-copper wastes on agricultural land. Contamination of the environment, whether by heavy metals or other waste products of society, has received considerable attention in recent years and with increasing public awareness, has become an emotive subject. It is therefore important that the findings of the present investigations with copper-rich pig slurry are evaluated carefully in the light of present knowledge of the environmental effects of heavy metal pollution from other sources and the economically important benefits of using copper as a growth promoter.

Although information regarding total useage of copper in pig rations in Great Britain is not available, the yearly output of copper in pig excreta has been estimated (Section 1.06, this thesis) to be in the region of 640 tonnes. If spread over the total area, 12 million hectares (C.S.O., 1979) which is under grass or crop in Britain, each hectare would receive on average 0.05 kg Cu per annum. From soil bulk density data given in Appendix I it may be assumed that there would be in the order of 2 million kg topsoil/ha, the total copper content of which would be increased by only 0.025 mg/kg/annum. This would appear to be a negligible increase in view of the fact that the average total copper content of uncontaminated topsoils has been reported (Vinogradov, 1959) to be in the region of 20 mg Cu/kg. However, the development of intensive systems of production has led to a situation where large volumes of pig slurry have to be disposed of on limited areas of land. The problem is particularly acute where large numbers of pigs are kept on small specialised holdings such that the quantities of excreta produced have a fertiliser value, with respect to N, P and K, far in excess of the requirement of the land available for grass or arable crop production.

Approximately 10% of the pig herds, accounting for 12% of the total pig population in England and Wales in 1975 were maintained on holdings less than 2 ha in size (M.A.F.F., 1977). In such situations the pig slurry produced cannot be used rationally as a fertiliser and according to Berryman (1970) slurry application to land may often exceed 448,000 litres/ha on some farms, resulting in the addition to the land of copper at rates of up to 50 kg Cu/ha. At such application rates the total copper content of many soils could be doubled in one year.

Owing to the low mobility of copper in soils, which is a reflection of the strength with which the element is bound to other soil constituents, precipitated or occluded in insoluble compounds (Hodgson, 1963, a review), copper added to the topsoil shows little movement out of this surface layer (Jones and Belling, 1967; Andersson, 1977). Continued additions of copper and indeed other heavy metals to soils (Lagerwerff, 1967) usually results in the accumulation of the element in the topsoil. The results of the present investigations have shown that repeated applications of copper-rich pig slurry to land results in enhanced concentrations in the soil, particularly the surface layer; approximately 90% of the slurry copper added over two grazing seasons was recovered by extraction with EDTA. Although virtually no information has been published regarding the accumulation of copper in soils as a result of pig slurry disposal, there is abundant evidence that copper, zinc and other heavy metals added year after year to soils as inorganic salts and in waste materials such as municipal composts and sewage sludges, may lead to substantial contamination of the soil (Reuther and Smith, 1954; Le Riche, 1968; Andersson and Nilsson, 1976).

The practice of disposing of large amounts of copper-rich pig slurry on the same small areas of land each year, as is the case with many intensive pig units on small holdings (Berryman, 1970), must therefore be viewed with concern. In addition to the build-up of the major nutrients N, P and K in the soil, copper may accumulate to levels greater than those tolerated by agricultural crops.

Purves (1977) has reported that phytotoxicity may occur in clovers when the concentration of EDTA extractable copper in soils is 30 mg Cu/kg or greater; oats however were found to be more tolerant, showing no growth reduction until soil EDTA-Cu concentrations of 100 mg Cu/kg are encountered. In the present investigations, application of slurry at rates supplying 4 times the maximum yearly N requirement of grass at each application (50,000 l/ha) increased the soil.EDTA-extractable copper to levels in excess of 20 mg/kg soil, a total of 22 to 29 kg Cu/ha having been added to the soil. Since copper added to soil cannot be removed by any practical method on a large scale, applying slurry at such rates would appear to be extremely unwise.

The simplest answer to the problem of copper contamination of the soil arising from the use of this element as a growth promoter would be to greatly reduce the amount of copper added to pig feeds. Clearly a reduction in the feeding level to 125 mg Cu/kg as stipulated in E.E.C. Secondary Legislation (1972) would do no more than double the period of time which would elapse before undesirable levels accumulated in soils.

In the case of pig units where as much as 448,000 l slurry are applied/ha/annum, this reduction in level of supplementation would only extend the period of accumulation from 1 to 2 years before copper added to soil in slurry would reach a level (30 mg EDTA-Cu/ha) known to be toxic to clovers.

Concern regarding heavy metal contamination of soils has resulted in the publication of recommendations for maximum tolerable additions of these elements to soil (Department of the Environment, 1977). The guidelines, although mainly concerned with the disposal of sewage sludges, are applicable to other wastes which are applied to land, including copper-rich pig slurry. The maximum allowable additions of zinc and copper to land are given as 560 kg Zn or 280 kg Cu/ha over a 30 year period when either element is applied on its own. No restrictions have been given as to the quantity which may be applied in any one year and the application of copper may be doubled to 560 kg Cu/ha/30 years (on average, 18.7 kg Cu/ha/year) if applied to grassland.

On the basis of N, P and K contents of slurries reported in this work (Appendix VI), slurry supplying 18.7 kg Cu/ha would provide the major nutrients at rates far in excess of those recommended for grassland (351 kg N, 35 kg P and 67 kg K/ha) by the Ministry of Agriculture Fisheries and Food (1973). Furthermore, since up to 90% of the slurry copper added to soil was found to be EDTA-extractable, such additions of copper would, in two years, increase EDTA-Cu concentrations to levels known to adversely affect the growth of clovers.

While it is not possible to predict from the present studies how such applications of slurry copper would affect crop growth if continued for thirty years, a number of points may be made regarding the short term effects of slurry application on soil copper content and the concentration of copper in herbage.

Application of copper-rich pig slurry increased the concentration of EDTA-extractable copper in the surface 0 to 15 cm layer of soil to levels in excess of 20 mg Cu/kg soil. The accumulation of copper in the surface horizons reflects the ability of soil constituents to retain this element, the distribution of the added slurry copper being similar to that reported by Andersson (1977) in studies on the retention and distribution of native copper in soils. The liquid phase extracted from copper-rich pig slurry was found to contain in excess of 4 mg Cu/l, in agreement with the values obtained by McGill et al.(1975) using a different fractionation procedure. Evidence was obtained indicating that the copper in this liquid fraction, when added to soil, could contribute to the copper taken up by ryegrass under greenhouse conditions. In support of the theory that the increased concentrations of copper in herbage, after field application of slurry, may be due in part to soluble copper in the liquid fraction, the laboratory studies demonstrated that this copper fraction may markedly increase soil solution plus exchangeable copper as extracted by 0.05 M $CaCl_2$ solution. Copper bound to mainly inorganic sites in soil as determined by acetic acid extraction (Mitchell et al, 1957a,b), was also increased by soluble copper in the liquid phase. Since these soil copper fractions have been considered readily available to plants (McLaren and Crawford, 1973a), enhanced concentrations of copper in herbage grown on slurry treated land should not be unexpected.

The observations on the desorption of copper from slurry solids and investigation of precipitates which formed in stored slurry solution suggested that copper may not be present as sulphide in slurry or dried slurry solids. If, as suggested, the slurry copper is bound to organic matter, it would be expected that this copper would be released during decomposition of the solids and thus enter into the equilibrium between the various forms of soil copper constituting a potential source of copper for plants over extended periods of time.

Application of slurry consistently increased herbage copper concentrations following field applications of copper-rich slurry. The maximum copper concentration in herbage, 24.4 mg Cu/kg D.M., following slurry application, was considerably greater than that of herbage in England and Wales which has been reported to range from 7.4 to 9.0 mg Cu/kg D.M. (M.A.F.F., 1974).

The effect of copper addition to soil on the concentration of the element in the aerial parts of plants may be influenced by a large number of interreacting factors (Hodgson, 1963). In the present studies the increase in copper content of white clover was markedly greater than that in perennial ryegrass following slurry application. Such species effects may be explained by differences in translocation from roots to tops, as evidence has now appeared suggesting that a specific chelator may be involved in copper translocation (Tiffin, 1972). The Gramineae appear to possess a very effective mechanism for restricting translocation of copper from roots to tops. Jarvis et al (1979) found that the copper content of ryegrass shoots was increased 4 fold while that of the roots was increased 200-fold when the concentration of copper in the solution culture medium was increased from 0.16 to 157 μM. The observation that the copper content of ryegrass did not show a continued increase with increasing amounts of slurry copper added to the soil may well have resulted from effective control over translocation of this element. This finding would suggest that extractable levels of copper in soils may be a very poor guide in the prediction of the effect of slurry copper on the concentration of copper in grasses.

In the present investigations, the effect of slurry application on copper content of ryegrass was greater on the imperfectly draining clay loam soil compared to the freely draining sandy soil and also in the young fast growing herbage compared to more mature herbage at ear-emergence stage; there was also a tendency for copper concentrations to increase towards the end of the growing season. Such soil, stage of maturity, and seasonal effects may be of little importance when viewed in relation to the ability of grass to limit copper translocation to the tops. Such translocation control may be beneficial in limiting the amounts of slurry copper entering into the food chain.

The copper concentrations observed in slurry treated herbage in this and other studies (Batey et al, 1972; Lawrence and Gibbs, 1973; Kneale and Smith, 1977) are considerably greater than the level, 15 mg Cu/kg D.M., in diets which have resulted in copper poisoning in sheep (Suttle, 1968; Buck, 1970). The lack of persistence of enhanced copper concentrations in herbage for periods greater than 4 to 5 weeks on slurry treated land would suggest that there may be little danger to sheep grazing such herbage with no surface contamination. The high availability of copper in dried slurry solids, an availability similar to that of copper in the form of copper sulphate, may well pose a more serious threat to grazing sheep or sheep maintained on conserved herbage contaminated with slurry solids. Dalgarno and Mills (1975) have concluded that ingestion of slurry solids at a level contributing 2% of the dry matter intake may constitute a hazard to sheep if consumed for periods in excess of 6 months. The inclusion of pig excreta at levels of up to 30% (Bhattacharya and Taylor, 1975) on a D.M. basis in rations for ruminants must therefore be undesirable in the light of present knowledge.

The risk of copper poisoning of farm livestock as a result of ingestion of slurry solids could be effectively reduced by grazing only mature cattle, which are relatively tolerant of high copper intakes (Underwood, 1971), on slurry treated land. Furthermore, slurry application could be carried out on closely grazed pasture or ploughed in for arable crops; the inclusion of high copper pig wastes in rations for ruminants, the most serious risk of all judging from the studies of Hendrosoekarjo and Pearce (1978), could be prohibited with little effect on the economics of livestock production.

Although Ross (1966) and Hogan et al (1968) have been successful in preventing copper poisoning in sheep fed high copper diets by supplementing the diet with molybdenum and sulphate to reduce copper retention, this method has not been applied to any extent under practical situations. Due to the difficulty of predicting both copper availability in a ration or herbage or of the effect of dietary additions of molybdenum and sulphur on availability or retention of the dietary copper, this method is unlikely to gain wide acceptance as a means of maintaining copper status at a satisfactory level in sheep with a high copper intake.

Supplementation of sheep or calf rations with 220 to 420 mg Zn/kg D.M. as suggested by Bremner et al (1976) may be an alternative method for reducing copper retention in sheep. However, the major disadvantage of both methods lies in the difficulty in supplementing livestock at grass, the period when they are probably most at risk from slurry contamination of herbage.

In the final analysis, the economic benefits of using copper as a growth promoter in pig fattening rations must be weighed against the soil pollution aspects and the resultant risk of toxicity to plants and animals. As yet there have been few reports of deaths (Loosemore, 1969; Feenstra and van Ulsen, 1973) in farm livestock which can be directly attributable to copper originating from pig slurry. Since the greatest risk appears to be associated with direct ingestion of slurry solids, the hazard to animals might be considered simply as a matter of management. More serious is the accumulation of slurry copper in soils due to continued disposal of copper-rich pig slurry as the copper once added to the soil cannot be removed. While it might be argued that soils have an extremely large capacity for fixation and retention of heavy metals such as copper on organic matter, manganese and iron oxide fractions (McLaren and Crawford, 1973b), continued additions of copper to soils must influence the equilibrium between the various soil copper fractions. Ultimately the concentration of copper in the soil solution may increase to levels which are phytotoxic. The irreversibility of copper contamination of soils (Purves, 1977) makes this a very undesirable situation; Patterson (1966) has reported that a market garden which had been treated with large amounts of sewage sludge can no longer be used for vegetable production.

Although the availability of heavy metals is reputedly lower when added to soils in organic materials than as inorganic salts,(Cunningham, Keeney and Ryan 1975), the major factor influencing slurry copper uptake by grasses, the main pasture species in Great Britain,would appear to be control over translocation. There would also appear to be little risk to grazing livestock from copper-rich pig slurry applied at rates consistent with crop requirements for the major nutrients N, P and K.

While biological treatment systems as described by Hobson and Robertson (1977) may reduce the organic and nitrogenous polluting capacity of pig wastes, copper is not bio-degradable and such treatment systems may enable intensive pig units on small holdings to continue adding large amounts of copper to the same land year after year. The solution to the copper problem which really only exists on a small proportion of farms, i.e. the intensive small holdings, in Great Britain, would appear to lie in biological treatment processes to reduce the bulk and polluting capacity of the excreta produced, facilitating transport to larger farms where the material may be used as a fertiliser.

Since recent surveys have revealed that appreciable numbers of cattle in Northern Ireland are hypocupraemic (Todd and Thompson, 1976) and that over 50% of the suckler cow herds in Wales are copper deficient (Davies and Baker, 1974) the dispersal of treated high-copper pig wastes to farms not producing pigs, may actually have a beneficial effect.

This thesis has been concerned solely with untreated pig slurry. In view of reports indicating a low availability to plants of copper in aerobically treated pig waste (Mutlak, 1974), in slurry stored under anaerobic conditions (Kneale and Smith, 1977) and the low apparent availability of slurry copper to sheep (Woodside, 1972) further research may be required to establish the forms of copper present in pig slurries under varying conditions.

APPENDIX I

Characteristics of soils from field sites

(Depth 7.5 to 15 cm)

Site	A, A1	B, B1
Association	Darvel	Rowanhill
Series	Darvel	Winton
Fine + Coarse Sand %	74.9	59.4
Silt %	8.9	13.5
Clay %	13.7	23.2
Carbon %	2.15	4.5
Bulk Density (g/ml)	1.4	1.3
pH	6.2	6.1
EDTA-Cu (mg/kg	2.2	6.2

Particle size distribution in the soil was determined by the method of Kilmer and Alexander (1949) and per cent carbon by the method of Walkley and Black (1934). Bulk density of the soils was determined on soil cores taken at a depth of 0 to 15 cm using a 7.5 cm diameter tubular core sampler; the soils being dried for four days at 40°C.

APPENDIX II

Procedures used in extraction of soil with 0.02 M EDTA and 1 N HCl

Soil samples were air dried, ground and the fraction passing a 2 mm screen retained for analysis. Soil pH was determined in a slurry of soil in distilled water (1:1 w/v) using a glass electrode and pH meter.

Extraction of soil with 0.2 M EDTA or 0.1 N HCl was carried out in wide necked polythene bottles with screw tops which were washed with the extractant before use. A 10 g aliquot of soil was shaken with 100 ml of 0.02 M EDTA (di-sodium salt, pH 4.2) for 1 hour and allowed to stand for 1 hour as suggested by Henriksen and Jensen (1958) or with 100 ml of 1 N HCl for 5 hours and allowed to stand for 1 hour. The extracts were filtered through Whatman No. 3 filter paper and copper concentration determined directly on the filtrate by atomic absorption spectrophotometry (Varian Tectron AA5). Standard solutions containing 0.2 to 1.6 mg Cu/l were prepared in 0.02 M EDTA or 1 N HCl solution. A shaking bottle containing extractant only was included in all batches of soils extracted.

Examination of the amount of copper extracted by 1 N HCl in relation to shaking time revealed that extraction times greater than 5 hours did not detectably increase the amount of copper extracted.

Four 10 g aliquots of 20 separate samples from each of the two soils described in Appendix I were weighed out and placed in extraction bottles; 10 samples from each soil type were from land which had received dressings of copper-rich slurry. A 10 g aliquot of each soil sample was extracted with 0.02 M EDTA and a further aliquot with 1 N HCl; copper sulphate solution (10 ml) containing 2.5 mg Cu/l was added to the remaining 2 aliquots before extraction.

The overall mean percent recovery ($\pm$ standard deviation) of added copper was 98.6 ± 1.06 for EDTA extracts and 99.2 ± 0.96 for HCl extracts.

APPENDIX III

Determination of total nitrogen, phosphorus and potassium concentrations in pig slurry and the liquid phase extracted from slurry

Total nitrogen in slurry or slurry extracts was determined on 1 to 2 g aliquots of liquid slurry or 3 ml aliquots of the liquid phase from slurry digested slowly in concentrated sulphuric acid using the Kjeldahl procedure of Jackson (1958).

Total phosphorus was determined by the vanado-molybdo-phosphate method described by Jackson (1958) and the concentration of potassium in these digests determined by flame emission spectrophotometry.

APPENDIX IV

Determination of the concentration of Cu, Zn, Mn and Fe in herbage and slurry

Herbage and slurry samples were dried at 80°C for 18 and 48 hours respectively in a forced draught, hot air oven and ground in a hammer-mill containing no copper or brass components. For determination of copper, zinc, manganese and iron, herbage and slurry dry matter, 2 g and 1 g respectively were ashed overnight in silica-glass crucibles at 450°C. Tne ash was taken up in 2 ml of 6 N HCl (Analar grade) and evaporated to dryness on a steam bath; this procedure was repeated once more. Finally, the ash was extracted with 5 ml of 1.5 N HCl on a steam bath and transferred through Whatman No. 30 filter paper into a volumetric flask using boiling distilled water. After filtering extracts of slurry ash, 5 ml of 6 N HCl were run onto the filter paper and the paper again washed with boiling distilled water. The extract from the ash of herbage was made up to 20 ml and that from slurry to 100 mls with a further 1 to 4 dilution.

The concentrations of Cu, Zn, Mn and Fe in extracts of the ash were determined by atomic absorption spectrophotometry (Varian Tectron, AA5). The instrument was calibrated using the following standard solutions:

copper;	concentration range	0.2	to	6.4	mg/l
zinc;	" "	0.2	to	6.4	"
manganese;	" "	1.0	to	32	"
iron;	" "	2.0	to	64	"

All standard solutions contained 1000 mg Ca/l, 2000 mg K/l and were 0.375 N with respect to HCl.

Solutions extracted from slurry were diluted 1 to 1 (v/v) with 0.75 N HCl and the concentration of copper in the acidified solution determined as for the ash extracts.

Standard additions of Cu, Zn, Mn and Fe to 10 samples of herbage dry matter (2 g) or dried slurry solids (1 g) before ashing gave the following percent recoveries ($\pm$ standard deviations) of the added elements on analysis:

	Herbage	Slurry solids
Cu	97.1 ± 1.5	96.8 ± 2.0
Zn	99.2 ± 0.8	98.8 ± 1.5
Mn	98.8 ± 1.8	97.8 ± 2.0
Fe	95.5 ± 2.2	96.2 ± 4.9

Typical sensitivities in determination of Cu, Zn, Mn and Fe using the Varian Tectron, AA5, with an air/acetylene flame, have been quoted by the makers as 0.04, 0.009, 0.024 and 0.062 mg/l respectively for these elements at the solution concentrations determined in the present studies.

In order to test for surface contamination of herbage with slurry, 20 samples from slurry treated swards were split into two sub-samples; the samples were obtained from the regrowth 4 weeks after each of 4 slurry applications. One sub-sample was steeped in distilled water for 20 minutes and rinsed with further distilled water 3 times before drying. Washed and unwashed sub-samples did not differ significantly in Cu, Zn, Mn or Fe content.

APPENDIX V

Determination of the concentration of copper in calcium chloride and acetic acid extracts of soil

Soil (25 g) was extracted with 250 ml of 0.05 N $CaCl_2$ or 0.5 N redistilled acetic acid using the procedure of McLaren and Crawford (1973a). The extracts were centrifuged at 1200 G for 10 minutes. Aliquots of 200 ml of $CaCl_2$ supernatant or 100 ml acetic acid supernatant were pipetted into 200 ml glass beakers, 2 ml of hydrogen peroxide (2 volume) added to destroy organic matter and the solution evaporated to dryness on a steam-bath. The sides of the beakers were washed down with 25 ml 6 N redistilled HCl, the beakers covered with watchglasses and the solutions heated on a steam-bath for 1 hour. The watchglasses were then removed and the solutions evaporated to dryness. The residue was taken up in 2 ml of 1 N redistilled HCl, giving solutions varying in concentration from 0.15 to 0.6 mg Cu/l ($CaCl_2$ extracts) and 0.8 to 2.0 mg Cu/l (acetic acid extracts).

The concentration of copper in these solutions was determined by aspirating directly into an atomic absorption spectrophotometer (Varian Tectron, AA5) using an air acetylene flame and chart recorder. Standard solutions of copper contained 0.1 to 2.0 mg Cu/l in 1 N HCl. As a blank, 200 ml of extractant alone, was carried through the complete process and extraction of each soil sample was replicated 4 times.

A copper sulphate solution (5 ml) containing 0.2 mg Cu/l was added to $CaCl_2$ and acetic acid extracts of 10 soils and the total copper concentration of these extracts determined. The mean percent recovery ($\pm$ standard deviation) of added copper from $CaCl_2$ extracts was 93.2 $\pm$ 2.4% and from acetic acid extracts 95.8 $\pm$ 1.6%.

APPENDIX VI

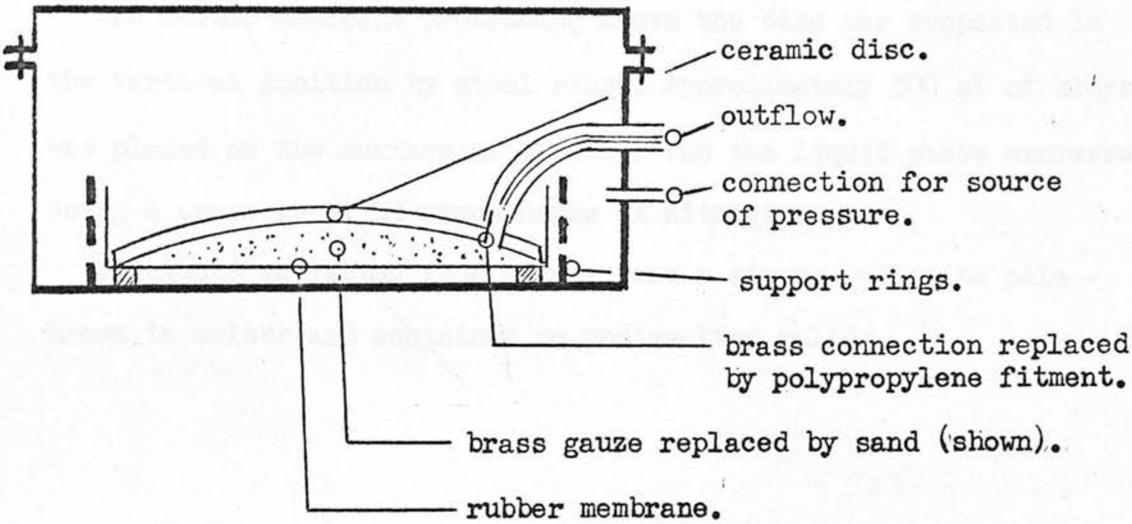
Composition of pig slurry and mineral fertiliser applied to established PRG sward (Means ± SD)

	SLURRY				MINERAL FERTILISER
Constituent	Site A1		Site A1		Sites A1, B1
	1972	1973	1972	1973	1972, 1973
N (g/100 g)	0.71 ± 0.02	0.47 ± 0.01	0.77 ± 0.03	0.49 ± 0.03	20.0
P "	0.23 ± 0.03	0.22 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	4.4
K "	0.29 ± 0.02	0.41 ± 0.03	0.30 ± 0.01	0.41 ± 0.03	8.3
D.M. "	8.30 ± 0.47	14.35 ± 0.65	10.08 ± 0.96	15.55 ± 0.70	100
Cu (mg/kg D.M.)	757 ± 21	542 ± 10	751 ± 18	564 ± 33	4.8 ± 0.01
Zn "	626 ± 15	490 ± 7	659 ± 12	485 ± 25	26.2 ± 1.22
Mn "	530 ± 12	435 ± 12	518 ± 11	432 ± 36	16.2 ± 0.32
Fe "	10,210 ± 316	7,909 ± 242	11,460 ± 320	8,171 ± 302	106.5 ± 3.67

Manufacturers figures.

APPENDIX VII

Modifications to equipment designed for determination of soil moisture tensions to allow extraction of the liquid phase from slurry.



The equipment consists of a slightly domed ceramic disc, with pore size of 0.19 microns. A rubber membrane is bound to the disc by a steel wire around the circumference (diagram above); this membrane is separated from the underside of the disc by a brass gauze.

A brass connecting tube allows the passage of liquid from the underside to the top side of the disc and hence externally via polythene capillary tubing. The complete disc is placed in a stainless steel container which may be pressurised using nitrogen from a compressed gas cylinder.

The brass connection tube was removed and replaced by a specially made polypropylene fitment. The brass gauze was removed and replaced with acid-washed sand(particle size 1 to 2 mm.), allowing liquid to move to the exit pipe.

The modified disc was soaked in 0.02M EDTA solution for 2 days and a volume of this solution forced through it until less than 0.05 mg Cu/l was detectable in the outflow. The disc was then washed further by passage of distilled water to remove all traces of EDTA.

The rubber membrane protruding above the disc was supported in the vertical position by steel rings. Approximately 500 ml of slurry was placed on the surface of the disc and the liquid phase expressed using a pressure of 15 atmospheres of nitrogen.

Solutions extracted from slurry were a strong yellow to pale brown in colour and contained no undissolved solids.

APPENDIX VIII

<u>Response in plasma copper of initially hypocupraemic ewes repleted by feeding herbage or a semi-synthetic diet containing $CuSO_4$ or dried pig slurry</u>

Week		Response in plasma Cu (mg/l) 1	2	3	4
Diet	Ewe No.				
UNTREATED HERBAGE	667	0.02	0.14	0.38	0.46
	476	0.04	0.08	0.28	0.40
	497	0.05	0.18	0.20	0.51
	320	0.03	0.15	0.46	0.52
	473	0.07	0.29	0.42	0.54
	506	0.04	0.31	0.49	0.55
	Mean ± S.E.	0.043 ± 0.008	0.19 ± 0.036	0.37 ± 0.046	0.50 ± 0.028
SLURRY TREATED HERBAGE	501	0.23	0.46	0.60	0.65
	679	0.20	0.43	0.67	0.73
	483	-0.03	0.25	0.57	0.70
	676	0.16	0.28	0.43	0.67
	672	0.13	0.68	0.77	0.86
	524	0.22	0.48	0.69	0.79
	Mean ± S.E.	0. 15 ± 0.040	0.43 ± 0.064	0.62 ± 0.048	0.73 ± 0.032

APPENDIX VIII (CONTINUED)

		Response in plasma Cu (mg/1)			
Week		1	2	3	4
Diet	Ewe No.				
S.S. DIET + $CuSO_4$	664	-0.07	0.03	0.21	0.29
	502	-0.06	0.12	0.31	0.36
	505	0.15	0.41	0.67	0.65
	669	0.01	0.06	0.20	0.30
	435	-0.06	-0.01	0.25	0.36
	659	0.06	0.13	0.39	0.52
	Mean ± S.E.	0.05 ± 0.036	0.12 ± 0.061	0.34 ± 0.072	0.41 ± 0.058
S.S. DIET + SLURRY	683	0.15	0.28	0.54	0.84
	433	-0.09	-0.05	0.17	0.21
	495	0.00	0.00	0.21	0.39
	485	0.05	0.07	0.20	0.35
	470	0.03	0.27	0.48	0.48
	507	-0.03	-0.04	0.11	0.21
	Mean ± S.E.	0.02 ± 0.033	0.09 ± 0.061	0.29 ± 0.073	0.41 ± 0.096

Initial plasma copper concentrations ranged from 0.13 to 0.28 mg Cu/1.

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The availability to sheep of copper in pig–slurry and slurry–dressed herbage. By J. PRICE, *Edinburgh School of Agriculture, Edinburgh* and N. F. SUTTLE, *Moredun Research Institute, Edinburgh*

The application to pasture of slurry from piggeries where copper is used as a growth stimulant constitutes a potential toxicity hazard to the grazing animal. In order to assess the risk of toxicity, the availability of Cu in slurry and slurry-dressed pasture to sheep was measured.

Slurry was obtained from a piggery where 150 mg supplementary Cu/kg food was used: it contained 425 mg Cu/kg dry matter (DM) and 220 g DM/kg and was applied at 15·6 tonnes/ha to part of a cut perennial ryegrass–white clover sward. Some slurry was retained and dried at 100° for 48 h. Two groups of six initially hypocupraemic Scottish Blackface ewes, 3 years old, were repleted for 28 d with 800 g/d of a semi-purified diet supplemented with Cu (8 mg/kg) as either $CuSO_4$ or dry slurry, while two groups were repleted with herbage (4 kg/d) from the dressed or undressed sward. Grass was given either fresh (day 0–18) or frozen (day 19–28). The first cut was taken 3 weeks after slurry application and herbage Cu and DM were determined on several cuts. Plasma Cu was measured weekly during repletion and Cu availability was predicted from the observed responses (Suttle, 1974).

The diets containing $CuSO_4$ and slurry produced similar responses (Table 1), indicating that slurry Cu is relatively available and potentially toxic when ingested as a pasture contaminant. The application of slurry to pasture increased herbage Cu from 7·3 to 10·2 mg Cu/kg DM in the first 12 d: thereafter, the differences narrowed to 0·5–1·9 mg/kg, indicating minimal leaf surface contamination. The response in plasma Cu was also increased ($P<0·001$) but enhanced availability contributed to the effect (Table 1).

Table 1. *Availability of copper in pig-slurry and slurry-dressed herbage predicted from the responses in plasma Cu of initially hypocupraemic ewes after 21 d repletion*

	Dietary Cu			
Cu source	Concentration (mg/kg DM)	Intake (mg)	Δ Plasma Cu (μg/l)	Cu availability* (%)
$CuSO_4$†	8·9	6·4	335±72	4·3
Dry slurry†	9·4	6·8	285±73	3·8
Grass	6·6	6·2	373±46	4·8
Slurry-dressed grass	8·5	7·0	620±48	5·8

DM, dry matter.
*Predicted by the technique of Suttle (1974).
†Added to low-Cu semi-purified diet.

The main risk in applying high-Cu slurry to pastures lies in the ingestion of contaminant Cu by the grazing animal: this risk can be minimized by treating cut swards, delaying grazing, and using cattle rather than sheep.

REFERENCE

Suttle, N. F. (1974). *Br. J. Nutr.* **32**, 395.